

NUTRITIONAL AND CHEMICAL ECOLOGY OF SELECT
NOCTUID CATERPILLARS WITH EMPHASIS ON
THE VELVETBEAN CATERPILLAR

By

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Gregory S. Wheeler

This dissertation is dedicated to my grandparents,
Sherman C. Wheeler and Ilma Hilton Wheeler for their love and
wisdom.

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Abstract of Dissertation Presented to the Graduate School
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By

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December 1989

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Major Department: Entomology and Nematology

Soybean foliage from susceptible and resistant lines was extracted in a number of organic solvents to assess constitutive (undamaged) and induced (damaged) effects. When incorporated in artificial diet, the benzene fraction contained the constitutive activity of both the resistant and susceptible lines, as indicated by reduced relative growth rate (RGR) of several noctuid soybean-adapted and non-adapted herbivore species. Induced resistance, detected only in the petroleum ether fraction, reduced RGR of the fall armyworm.

Caterpillars of the fall armyworm reared on artificial diets diluted with cellulose and water, increased fresh weight (fw) consumption 2.5-fold over those on undiluted diet. At the moderate levels of water- or cellulose-

dilution, this increased consumption, combined with increased digestion and absorption of nutrients (ADNU), sufficiently compensated for the reduced nutrient intake to achieve pupal biomass equivalent to that on the undiluted diet. At higher levels of water- and cellulose-dilution, fw consumption and ADNU increased further but pupal dry weight declined on the water-diluted diets. At each level of dilution, fw consumption and ADNU increased similarly on the water- and cellulose-diluted diets, but weight gain was reduced on the water- compared with the cellulose-diluted diets. This was due in part to lowered food conversion efficiency on the water-diluted diets, possibly caused by increased costs of metabolizing the wetter diets. My data support the hypothesis that consumption rates are regulated by nutrient feedback and possibly further modified by volumetric feedback mechanisms. The cost of increased consumption rates on diets of reduced energetic value may constitute a more significant energy expenditure than previously believed.

The adaptive significance of this compensatory feeding was examined in terms of avoiding ingestion of a lethal dose of allelochemical. Velvetbean caterpillars were fed sublethal doses of the benzene fraction in water-diluted diet. The increased feeding that occurred on extract-containing, diluted diets, was less than that on the extract-free diets. This difference was probably caused by

the toxic impact of the ingested compounds, possibly interacting with dietary water, rather than being an adaptive response.

CHAPTER 1 GENERAL INTRODUCTION

Nutritional Ecology of Insect Herbivores

The paradigm of nutritional ecology (Slansky and Rodriguez 1987) implies that an insect has a genetically programmed suite of performance values (e.g., body size, developmental rate, fecundity) that is attained under ideal environmental conditions. The environment, however, is rarely ideal; consequently, natural environments place constraints on the consumption, utilization and allocation of food, altering performance values accordingly. Insect herbivores have evolved the ability to perceive the conditions of the natural environment that alter their performance and to respond in an adaptive manner. These responses usually involve modifications in the consumption, utilization and allocation of food, along with the associated behavioral and physiological changes. These changes may involve inductive (e.g., diapause in response to decreasing day length) or compensatory (e.g., increased consumption on a dilute diet) responses that mitigate the negative impact of the environment. Thus, there are trade-offs between the costs and benefits of these adaptive responses; the costs generally include reduced performance

and the associated decrease in fitness, whereas the benefits of the response may be assessed in terms of survival or production of viable offspring. The combination of the nature and degree of the environmental constraints, the species' preprogrammed performance values and their ability to perceive and respond adaptively to environmental constraints select for a diversity of herbivore lifestyles.

Knowledge of the factors influencing the rate of food consumption and its efficiency of utilization may provide a better understanding of herbivores' ability to respond to environmental constraints. Non-nutritional dietary components (allelochemicals) that alter non-adapted herbivore performance or behavior (e.g., toxins, repellents) may reduce growth either directly by inhibiting primary metabolic pathways or indirectly by reducing consumption and the utilization of the ingested food. Allelochemicals to which an insect is adapted may be detoxified (Ahmad et al. 1986), tolerated (Berenbaum 1986), used as a host finding cue (Schoonhoven 1972), sequestered for protection from natural enemies (Duffey 1980), or may serve as sources of nutrients (Rosenthal et al. 1977). Reduced nutrient intake through either nutrient complexes with allelochemicals (Rhoades and Cates 1976) or incomplete or unbalanced composition may similarly alter herbivore feeding behavior (Waldbauer and Friedman 1988).

Dissertation Overview

This dissertation is divided into chapters designated for individual publications. Chapter 2 constitutes a review and synthesis of the pertinent literature regarding legume phylogenetics, allelochemistry and the plant species utilization by the velvetbean caterpillar Anticarsia gemmatalis Hübner. The third chapter includes experimental laboratory results of the extraction of constitutive (existent) and inducible soybean foliar allelochemicals and their influence on unadapted and adapted soybean herbivores. Chapter 4 describes further laboratory studies dealing with the compensatory abilities of the fall armyworm Spodoptera frugiperda (J. E. Smith) to water- and cellulose-diluted diets. Although incorporating distinct species of herbivores into a single dissertation may seem diffuse, each chapter fits into the overall body of research generated and in progress in the nutritional ecology research group.

CHAPTER 2
CHEMICAL ECOLOGY OF ANTICARSIA GEMMATALIS

Coevolution of Herbivores and Host Plants

Traditionally, secondary plant compounds were considered waste products of primary metabolism and of no benefit to the plant (Harborne 1982). This paradigm was questioned by Fraenkel (1959), who suggested that secondary metabolites provide plant defense against herbivores and that they are responsible for the patterns of herbivore-host selection. Ehrlich and Raven (1964) proposed a stepwise coevolution of plants and butterflies in which secondary plant metabolites select for a diverse pattern of host-plant utilization. These metabolites, or allelochemicals, may reduce the palatability of a plant or be otherwise deleterious to herbivore feeding. In response to these forces, the herbivores have evolved methods of countering the chemical defenses through a variety of means, among them avoidance of the toxic plant tissue (Mullin 1986), detoxifying the compounds (Ahmad et al. 1986) or developing tissue insensitivity (Berenbaum 1986). Some well-adapted herbivore species may use allelochemicals as cues for host location (Schoonhoven 1972), whereas others may sequester the compounds for their own protection from natural enemies

(Duffey 1980). Other well adapted herbivores may use allelochemicals as nutrients that are otherwise toxic or deterrent to non-adapted species (Rosenthal et al. 1977, Bernays and Woodhead 1982). In response to the breached plant defenses, the plants diversified the defenses, producing a diverse array of compounds with a variety of effects on herbivores.

This chapter includes a compilation of information regarding the velvetbean caterpillar Anticarsia gemmatalis Hübner larval host range and the associated plant allelochemistry. The purpose of this synthesis is to attempt to determine if A. gemmatalis larval host plant range exhibits any pattern with regard to legume systematics or allelochemistry. Included here is a compilation of data on larvae fed plant species in laboratories, reports of field records of larvae and a list of the natural products identified in potential host and non-host species. This information may assist researchers (e.g., plant breeders) interested in identifying possible chemical factors that may impart A. gemmatalis resistance in legume agronomic or horticultural crops. Thus, I will first propose a scheme of host utilization through the tribes of the Leguminosae, then review the literature describing the presence/absence of the classes of natural products reported from the A. gemmatalis potential hosts or non-hosts and finally relate these compounds to the potential larval host range. Other

pertinent reviews appear elsewhere: A. gemmatalis general life history and pest population management (Herzog et al. unpubl.); the physiological ecology of A. gemmatalis (Hammond and Fescemyer 1987) and the chemical mechanisms of soybean Glycine max (L.) Merrill resistance to herbivores (Smith 1985).

The Anticarsia Species

The velvetbean caterpillar (A. gemmatalis) is distributed throughout much of the subtropical and tropical new world and seasonally, moths migrate into temperate areas (Herzog and Todd 1980). Within this range the only area where A. gemmatalis has not been reported includes the Amazon basin. In addition to A. gemmatalis, 12 other Anticarsia species exist worldwide. These have been reported from Venezuela (A. acutilinea Walker), Ghana (A. albilineata Hampson), Honduras (A. anisospila Walker), Peru (A. disticha Hampson), Australia (A. distorta Hampson), Cuba (A. elegantula Herrich-Schäffer), India (A. irrorata Fabricius), Brazil (A. parana Guenée), China (A. renipunctum Berio, A. tigris Berio), Mexico (Veracruz) (A. suffervens Dyar), Cameroon (A. unilineata Gaede) (Poole 1989). The species A. repugnalis Hübner reported from south Florida (Tietz 1972) was changed to Azeta repugnalis (Hübner) (Poole 1989).

Apart from A. gemmatalis, considered a legume specialist (see below), information on host-plant utilization by these other species was recorded only for A. irrorata, with larvae were collected on the following legume species: Phaseolus mango; Doclichos (sic) lablab (Poole 1989); Arachis hypogaea; Medicago sativa; Canavalia bonariensis and Wisteria sinensis (Biezanko et al. 1957). Although the host range data regarding the entire genus are incomplete, they suggest that other Anticarsia species may also be legume specialists.

General Systematics of the Leguminosae

The Leguminosae comprises 650 genera and 18,000 species and constitutes the third largest family of flowering plants after the Compositae and Orchidaceae (Polhill et al. 1981). The family is divided into three, more or less distinct subfamilies, the Caesalpinioideae, Mimosoideae and Papilionoideae. The general divergence pattern of the tribes within the Phaseoloideae is presented in Fig. 2-1. The tribes of this subfamily originated from the ancestral Swartzieae and diverged, producing the Sophoreae and subsequently many radiating groups (Polhill 1981).

Host Utilization by A. gemmatalis

The data used in this chapter were compiled from a variety of published and unpublished sources (Table 2-1). Earlier reviews covered primarily field host records (Herzog

Fig. 2-1. Phylogenetic representation of the divergence of the tribes of the Papilionoideae (from Polhill 1981). Large numbers represent the predominant chromosome base number.

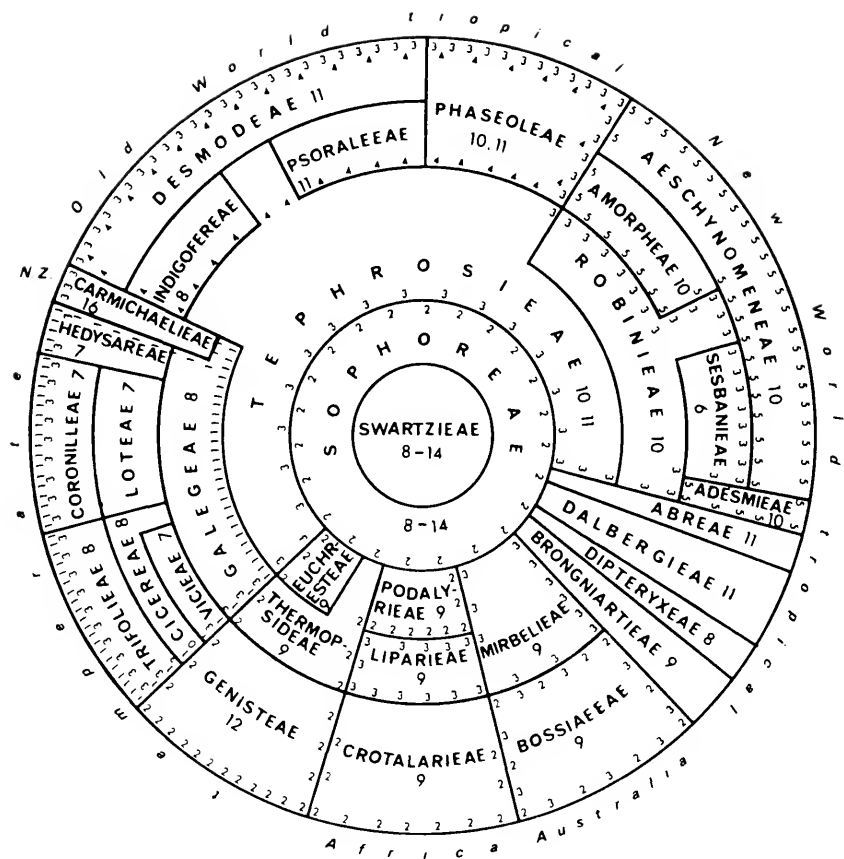


Table 2-1. Compilation of legume taxonomy and laboratory rearing data of larval A. gemmatalis survival. Taxa arranged phylogenetically after Polhill & Raven (1981).

Scientific name	Tribe	Subtribe ¹	Surv. ² %	Reference
Caesalpinioideae				
<u>Parkinsonia aculeata</u> L.	Caesalpinieae		NH	Slansky (1989)
<u>Chamaecrista fasciculata</u> (Michx.) Greene	Cassieae	Cassiinae	0	Cohen (unpubl.)
(= <u>Cassia fasciculata</u> Michx.)				
(= <u>C. chamaecrista</u> Kuntz)				
<u>Chamaecrista nictitans</u> (L.) Moench.	Cassieae	Cassiinae	0	Cohen (unpubl.)
(= <u>Cassia nictitans</u> L.)				
<u>Senna obtusifolia</u> (L.) I. & B.	Cassieae	Cassiinae	0	Cohen (unpubl.)
(= <u>Cassia obtusifolia</u> L.)				
<u>Senna occidentalis</u> (L.) Link	Cassieae	Cassiinae	0	Cohen (unpubl.)
(= <u>Cassia occidentalis</u> L.)				
<u>Cercis canadensis</u> L.	Cercideae	Cercidinae	20	Plagens (unpubl.)
			0	Plagens (unpubl.)

¹ Subtribes are listed only if they distinguish among members of the same tribe.

² Percent survival from laboratory rearings are noted numerically. Field host or non-host data are presented with the abbreviations 'H' or 'NH', respectively. If larvae were reported on a plant species that was regarded as a secondary host relative to other species, a '+' is used.

Table 2-1--continued.

Scientific name	Tribe	Subtribe	Surv. %	Reference
<u>Mimosoideae</u>				
<u>Schrankia</u>	Mimoseae		NH	Slansky (1989)
<u>microphylla</u> (Dry.) Macbr.			0	Plagens (unpubl.)
<u>Albizia</u>	Ingeae		0	Plagens (unpubl.)
<u>julibrissin</u> Durazz.				
<u>Papilionoideae</u>				
<u>Tephrosia</u>	Tephrosieae		90	Plagens (unpubl.)
<u>florida</u> (Dietr.) Wood			H	Slansky (1989)
<u>Wisteria</u> sp.	Tephrosieae		0	Plagens (unpubl.)
<u>Wisteria</u>	Tephrosieae			
<u>frutescens</u> (L.) Poir.	Robinieae		100	Plagens (unpubl.)
<u>Robinia pseudoacacia</u> L.			90	Plagens (unpubl.)
			H	Ellisor (1942)
			H	Anazonwu & Johnson (1986)
			70	Plagens (unpubl.)
<u>Sesbania</u>	Sesbanieae		100	Waters & Barfield (in press)
<u>emerus</u> (Aubl.) Urban	Sesbanieae			
<u>Sesbania</u>				
<u>exaltata</u> (Raf.) Rydb.				
(=Sesbania)				
<u>macrocarpa</u> Muhlenb.)				
<u>Sesbania</u>				
<u>grandiflora</u> (L.) Poir.	Sesbanieae		H	Wolcott (1936)
[=Agati]				
<u>grandiflora</u> (L.) Desv.]				

Table 2-1--continued.

Scientific name	Tribe	Subtribe	Surv. %	Reference
<u>Sesbania</u>	Sesbanieae		90	Plagens (unpubl.)
<u> punicea</u> (Cav.) Benth.				
<u>Sesbania</u>	Sesbanieae		0	Cohen (unpubl.)
<u> vesicaria</u> (Jacq.) Ell.			90	Plagens (unpubl.)
<u>Indigofera</u>	Indigoferaeae		80	Plagens (unpubl.)
<u> caroliniana</u> Mill.			100	Plagens (unpubl.)
<u>Indigofera hirsuta</u> L.	Indigoferaeae		100	Cohen (unpubl.)
			100	Plagens (unpubl.)
			H	Buschman <u>et al.</u> (1977)
			87	Conti & Waddill (1982)
			H	Cookman <u>et al.</u> (1984)
			95	Waters & Barfield (in press)
			H	Buschman <u>et al.</u> (1977)
<u>Desmodium</u>	Desmodieae	Desmodiinae		
<u> floridanum</u> Chapm.				
<u>Desmodium</u>	Desmodieae	Desmodiinae	0	Plagens (unpubl.)
<u> paniculatum</u> (L.) DC.				
<u>Desmodium</u>	Desmodieae	Desmodiinae	<5	Cohen (unpubl.)
<u> tortuosum</u> (Sw.) DC.			40	Plagens (unpubl.)
<u>Lespedeza</u>	Desmodieae	Lespedezinae	82	Waters & Barfield (in press)
<u> stipulacea</u> Maxim				
<u>Lespedeza striata</u>	Desmodieae	Lespedezinae	80	Plagens (unpubl.)
(Thunb.) Hok. & Arn.				
<u>Lespedeza</u>	Desmodieae	Lespedezinae	100	Plagens (unpubl.)
<u> hirta</u> (L.) Hornem.				
<u>Erythrina herbacea</u> L.	Phaseoleae	Erythrininae	0	Cohen (unpubl.)
			0	Plagens (unpubl.)
			0	Plagens (unpubl.)
			NH	Slansky (1989)

Table 2-1--continued.

Scientific name	Tribe	Subtribe	Surv. %	Reference
<u>Mucuna deeringiana</u> (Bort.) Merrill (=Stizolobium deeringianum Bort.)	Phaseoleae	Erythrininae	H	Watson (1916)
<u>Apios americana</u> Medic.	Phaseoleae	Erythrininae	0	Plagens (unpubl.)
<u>Canavalia gladiata</u> (Jacq.) DC.	Phaseoleae	Diocleinae	H	Ellisor (1942)
<u>Canavalia maritima</u> (Aubl.) Thouars (=Canavalia rosea Sw.)	Phaseoleae	Diocleinae	H	Buschman et al. (1977) Tietz (1972)
<u>Canavalia</u> sp.	Phaseoleae	Diocleinae	H	Watson (1916)
<u>Pachyrhizus erosus</u> (L.) Urban	Phaseoleae	Diocleinae	H	Ellisor (1942)
<u>Galactia spiciformis</u> T. & G.	Phaseoleae	Diocleinae	±	Buschman et al. (1977)
<u>Galactia elliptica</u> Nutt.	Phaseoleae	Diocleinae	±	Buschman et al. (1977)
<u>Galactia regularis</u> (L.) BSP.	Phaseoleae	Diocleinae	70	Plagens (unpubl.)
<u>Galactia volubilis</u> (L.) Britt.	Phaseoleae	Diocleinae	80	Plagens (unpubl.)
<u>Pueraria lobata</u> (Willd.) Ohwi (=Pueraria thumbergiana (Siebold & Zucc.) Benth.	Phaseoleae	Diocleinae	90	Plagens (unpubl.)
<u>Pueraria phaseoloides</u> (Roxb.) Benth.	Phaseoleae	Glycininae	80	Plagens (unpubl.)
<u>Glycyne max</u> (L.) Merrill	Phaseoleae	Glycininae	H	Watson (1916)
			H	Buschman et al. (1977)
			H	Anazomwu & Johnson (1986)
			H	Ford et al. (1975)
			95	Cohen (unpubl.)

Table 2-1--continued.

Scientific name	Tribe	Subtribe	Surv. %	Reference
(= <u>Phaseolus max</u>)				
<u>Centrosema</u>			100	Plagens (unpubl.)
<u>virginianum</u> (L.) Benth.			80	Plagens (unpubl.)
<u>Dolichos lablab</u> L.			79	Conti & Waddill (1982)
[= <u>Lablab</u>			49	Slansky (1989)
<u>purpureus</u> (L.) Sw.]	Phaseoleae	Clitoriinae	0	Plagens (unpubl.)
<u>Vigna</u>				
<u>luteola</u> (Jacq.) Benth.	Phaseoleae	Phaseolinae	H	Buschman <u>et al.</u> (1977)
[= <u>Vigna</u>			H	Guthrie (1977)
<u>repens</u> (L.) Kuntze]			73	Conti & Waddill (1982)
<u>Vigna umbellata</u>			H	Buschman <u>et al.</u> (1977)
(Thurnb.) Ohwi & Ohashi	Phaseoleae	Phaseolinae	73	Conti & Waddill (1982)
(= <u>Phaseolus</u>				Gregory <u>et al.</u> (in press)
<u>calcaratus</u> Roxb.)	Phaseoleae	Phaseolinae	H	Gutierrez & Pulido (1978)
<u>Vigna</u>				
<u>unquiculata</u> (L.) Walp.	Phaseoleae	Phaseolinae	H	Hinds & Osterberger (1931)
[= <u>Vigna sinensis</u> (L.) Endl.]			50	Plagens (unpubl.)
			70	Plagens (unpubl.)
			H	Nilakhe & Chalfant (1982)
			83	Waters & Barfield (in press)
<u>Macroptilium</u>				
<u>lathyroides</u> (L.) Benth	Phaseoleae	Phaseolinae	H	Ellisor & Graham (1937)
(= <u>Phaseolus lathyroides</u> L.)			H	Buschman <u>et al.</u> (1977)
<u>Phaseolus limensis</u> Macf.	Phaseoleae	Phaseolinae	87	Conti & Waddill (1982)
	Phaseoleae	Phaseolinae	H	Ford <u>et al.</u> (1975)

Table 2-1--continued.

Scientific name	Tribe	Subtribe	Surv. %	Reference
<u>Phaseolus lunatus</u> L.	Phaseoleae	Phaseolinae	<5	Cohen (unpubl.)
<u>Phaseolus semierectus</u>	Phaseoleae	Phaseolinae	H	Tietz (1972)
<u>Phaseolus speciosus</u> HBK.	Phaseoleae	Phaseolinae	±	Buschman <u>et al.</u> (1977)
<u>Phaseolus vulgaris</u> L.	Phaseoleae	Phaseolinae	10	Plagens (unpubl.)
			27	Conti & Waddill (1982)
<u>Cajanus</u>	Phaseoleae	Cajaninae	H	McCord (1974)
<u>cajan</u> (L.) Druce			H	Cookman <u>et al.</u> (1984)
(= <u>Cajanus indicus</u>)				
<u>Rhynchosia cinerea</u> Nash	Phaseoleae	Cajaninae	100	Plagens (unpubl.)
<u>Rhynchosia</u>	Phaseoleae	Cajaninae	90	Plagens (unpubl.)
<u>minima</u> (L.) DC.			±	Buschman <u>et al.</u> (1977)
			84	Conti & Waddill (1982)
<u>Rhynchosia</u>	Phaseoleae	Cajaninae	0	Plagens (unpubl.)
<u>reniformis</u> DC.			0	Plagens (unpubl.)
<u>Dalea pinnata</u>	Amorpheae			
(Walt. ex Gmel.) Barneby				
<u>Aeschynomene</u>	Aeschy.	Aeschin. ³	50	Plagens (unpubl.)
<u>americana</u> L.			H	Bullock & Kretschmer (1982)
<u>Aeschynomene</u>	Aeschy.	Aeschin.	0	Plagens (unpubl.)
<u>viscidula</u> Michx.				
<u>Arachis hypogaea</u> L.	Aeschy.	Stylo.	95	Cohen (unpubl.)
			80	Plagens (unpubl.)
<u>Chapmannia</u>	Aeschy.	Stylo.	0	Plagens (unpubl.)
<u>floridana</u> T. & G.	Galegae	Astragalinae	100	Plagens (unpubl.)
<u>Astragalus</u>				

³ Aeschy.=Aeschynomeneae; Aeschin.=Aeschynomeninae; Stylo.=Stylosanthinae.

Table 2-1--continued.

Scientific name	Tribe	Subtribe	Surv. %	Reference
<u>villosus</u> Michx.				
<u>Vicia angustifolia</u> L.	Vicieae		60	Plagens (unpubl.)
			90	Plagens (unpubl.)
			13	Slansky (1989)
<u>Lathyrus</u> sp.	Vicieae		10	Plagens (unpubl.)
<u>Lathyrus</u> sp. (Cult. var.)	Vicieae		20	Plagens (unpubl.)
<u>Lens culinaris</u> Medic.	Vicieae		0	Plagens (unpubl.)
<u>Pisum sativum</u> L.	Vicieae		40	Plagens (unpubl.)
			70	Plagens (unpubl.)
<u>Cicer arietinum</u> L.	Cicereae		60	Plagens (unpubl.)
<u>Melilotus alba</u> Desr.	Trifolieae		80	Plagens (unpubl.)
			H	Waddill (1981)
			56	Conti & Waddill (1982)
			68	Slansky (1989)
			96	Waters & Barfield (in press)
<u>Medicago lupulina</u> L.	Trifolieae		100	Plagens (unpubl.)
			42	Slansky (1989)
<u>Medicago sativa</u> L.	Trifolieae		H	Ellisor & Graham (1937)
<u>Trifolium repens</u> L.	Trifolieae		90	Plagens (unpubl.)
			81	Slansky (1989)
<u>Crotalaria</u>	Crotalarieae		0	Cohen (unpubl.)
<u>lanceolata</u> E. Mey.			0	Plagens (unpubl.)
<u>Crotalaria retusa</u> L.	Crotalarieae		40	Plagens (unpubl.)
<u>Crotalaria</u>	Crotalarieae		0	Cohen (unpubl.)
<u>spectabilis</u> Roth			0	Plagens (unpubl.)
			0	Waters & Barfield (in press)
<u>Baptisia</u>	Thermopsideae		70	Plagens (unpubl.)

Table 2-1--continued.

Scientific name	Tribe	Subtribe	Surv. Reference %
<u>lactea</u> (Raf.) Thiret (= <u>Baptisia</u> <u>leucantha</u> T. & G.) <u>Lupinus villosus</u> Willd.	Genisteae		40 Plagens (unpubl.)
Begoniaceae			
<u>Begonia</u> sp.			H Herzog & Todd (1980)
Geraniaceae			
<u>Geranium carolinianum</u> L.			0 Plagens (unpubl.)
Gramineae			
<u>Oryza sativa</u> L.			H Herzog & Todd (1980)
<u>Triticum aestivum</u> L.			H Herzog & Todd (1980)
Malvaceae			
<u>Gossypium herbaceum</u> L.			H Herzog & Todd (1980)
<u>Hibiscus esculentus</u> L.			H Herzog & Todd (1980)
Rosaceae			
<u>Licania michauxii</u> Prance			10 Plagens (unpubl.)
<u>Licania michauxii</u> Prance			0 Plagens (unpubl.)
<u>Prunus serotina</u> Ehrh.			0 Plagens (unpubl.)

and Todd 1980, Moscardi 1979, Gregory 1986, Gregory et al. in press). Laboratory rearing data are emphasized here, with virtually all methods involving the non-choice feeding on plant foliage by laboratory strains of A. gemmatalis to determine larval survival. In a few cases larval consumption, feeding efficiencies and development were recorded (e.g., Slansky 1989, Waters and Barfield in press). However, all the laboratory studies included percent caterpillar survival, and therefore this is the larval performance parameter used to compare the suitability of the different potential host species. A plant on which less than half the individuals reared survived is considered a non-host, as supported by data for well known host plants G. max, Indigofera hirsuta and Melilotus alba. This value was arbitrarily chosen and may be too conservative considering in some cases laboratory survival was only 49% on G. max foliage, a very common A. gemmatalis host plant (Slansky 1989).

Field and laboratory records are supportive of one another but both should be treated with caution as they may not directly overlap (Wiklund 1982). The field data may include plant species on which caterpillars were collected following host devastation, although oviposition occurred on a different field host. Field data may also include what may be ovipositional mistakes on plant species poorly utilized by larvae. On the other hand, laboratory results

imply consumption and utilization under very unnatural conditions where no choice in host plant species is available. Also the condition (e.g., phenophase, fitness, fertilization) of the plants used in the laboratory study may be different from the field plants utilized. Furthermore, the laboratory insect strains, often inbred for many years and from single sources, may not be representative of field populations in terms of tolerance to host plant allelochemicals or nutrition (Pashley 1986). Ovipositional data of female A. gemmatalis also are valuable but are rather scarce (Gregory et al. in press). Ideally, the combination of all three types of data (laboratory, field and ovipositional) would be most useful in determining the A. gemmatalis natural host range, but they have yet to be recorded extensively.

All reported host plants from field observations occur in the Leguminosae except single records from the malvaceous Gossypium hirsutum and Hibiscus esculentus, the Begoniaceae Begonia sp. and the two host records on rice and wheat (Gramineae; Herzog and Todd 1980); however all are regarded with suspicion (Gregory et al. in press). The results of the bioassays with non-legumes suggest that only members of the Leguminosae support A. gemmatalis growth and development.

Caesalpinioideae and Mimosoideae

All Caesalpinioideae and Mimosoideae species tested (Table 2-1) failed to support larval growth and development. These species included (% survival in parentheses): Parkinsonia aculeata (NH); Chamaecrista fasciculata (0%); C. nictitans (0%); Senna obtusifolia (0%); S. occidentalis (0%); Schrankia microphylla (0%), and Albizia julibrissin (0%).

Papilionoideae

Larvae of A. gemmatilis were reared successfully on only some of the tested species of the Papilionoideae. Non-hosts within this subfamily include (% survival): Aeschynomene viscidula (0%); Apios americana (0%); Centrosema virginianum (0%); Cercis canadensis (0-20%); Chapmania floridana (0%); Crotalaria lanceolata (0%); C. retusa (40%); C. spectabilis (0%); Dalea pinnata (0%); Desmodium paniculatum (0%); D. tortuosum (< 5-40%); Erythrina herbacea (0%); Lathyrus sp. (10-20%); Lens culinaris (0%); Lupinus villosus (40%); Phaseolus lunatus (< 5%); P. vulgaris (10-27%); Rhynchosia reniformis (0%); Sesbania vesicaria (0-90%); Vicia angustifolia (13-90%); Wisteria frutescens (0%).

Association of Herbivore and Host Species

The Leguminosae is believed to have originated primarily in Africa and diversified further in South America

(Raven and Polhill 1981). The phylogeny of the Papilionoideae tribes is depicted in Fig. 2-1, which indicates that all tribes diverged from the ancestral Swartzieae and Sophoreae (Polhill 1981). Compilation of laboratory feeding data (Table 2-1) suggests the association between A. gemmatalis caterpillars and their host species may be traced phylogenetically back to the tribe Tephrosieae (Fig. 2-1). The geographic distributions of Papilionoideae tribes in Fig. 2-1 (e.g., new world tropical) is believed to be their regions of origin and is not intended to represent their current ranges (Polhill 1981).

No feeding data are available for species of the most ancestral tribes, Swartzieae or Sophoreae; one visual search in Texas of Sophora tomentosa failed to reveal A. gemmatalis larvae (Gregory et al. in press). Two to three species of the Tephrosieae were tested, Tephrosia florida, Wisteria frutescens and Wisteria sp. Larval survival was 90% on T. florida, but no larvae survived on W. frutescens, whereas Wisteria sp. (possibly a different species) was listed as a potential host. Thus, A. gemmatalis caterpillars apparently continue to maintain the ability to utilize at least some of these possibly ancestral host species.

New World Tropical Tribes

Following the New World tropical tribes (Fig 2-1), A. gemmatalis larvae successfully completed development on

nearly all the Robinieae species with the exception of Sesbania vesicaria; however, many other members of this genus were acceptable hosts. The single member of the Amorpheae tested was not a host, while half of the four Aeschynomeneae species tested (one of each subtribe) were suitable hosts. Thus, the host range in the neotropics apparently extends through all the tribes.

Old World Tropical Tribes

Four Old World tropical tribes diverged directly from the Tephrosieae. One, Phaseoleae, is represented by 25 species in these feeding trials, of which six (24%) did not support A. gemmatilis caterpillar growth and development. These included two of the three Erythrinae, Erythrina spp. and Apios spp., two species of Phaseolus, Centrosema sp., and one of the three Rhynchosia species (one of the five Cajaninae) tested. With the possible exceptions of the Erythrina and Phaseolus, whose allelochemistry is reviewed below, most genera of the Phaseoleae served as hosts and the species that did not probably represent divergences from the general pattern of host suitability.

Additional Old World tropical tribes include the Indigoferaeae, Psoraleeae (not represented here by feeding trials) and the Desmodieae. Both of the two species tested of the Indigoferaeae served as hosts, while two of the four Desmodieae species (both Desmodium spp.) did not.

Although A. gemmatalis larval survival was relatively high when reared on three Lespedeza species (80-100%), larval developmental time was significantly greater and larval growth was reduced (both absolute growth rate and relative growth rate, with up to 10 molts required compared with the typical 5-7) relative to most other host species assayed (Waters and Barfield in press).

Temperate Tribes

The temperate Papilionoideae tribes extend primarily from the Tephrosieae through the Galegeae and into (for our survey) the Vicieae, Cicereae and Trifolieae. Only a single member of the Galegeae was tested (Astragalus villosus) and all larvae tested survived (100%). Two of the four species of the Vicieae tested supported A. gemmatalis growth and development. Larval survival when reared on foliage Vicia angustifolia was high in one study (60-90%, Plagens unpubl.) but low in another (13%, Slansky 1989), suggesting that variation in plant material may influence the acceptability of this species. The sole member of the Cicereae and all four members of the Trifolieae served as hosts.

Another divergence of temperate species radiates directly from the Sophoreae. The Thermopsidae is believed to be ancestral to the Genisteae and A. gemmatalis rearing data suggest that species of both tribes serve as hosts. The single species tested from each tribe supported A.

gemmatalis larval growth and development. Thus, A. gemmatalis larval feeding specialization may be traced in the temperate zone from members of the ancestral Papilionoideae tribe, Tephrosieae, through the Thermopsidae and Galegeae, to the more advanced Vicieae, Cicereae, Trifolieae and Genisteae.

The remaining tribes originated in New Zealand, Australia and Africa. Although no legume species from New Zealand or Australia were tested, data are available on five species of a single African tribe, the Crotalarieae. The rearing data suggest that all members of this tribe, with the possible exception of Crotalaria retusa (40% survival), are non-hosts. This may be due to the phylogenetically isolated position of the tribe, with no known links to the ancestral host tribes and/or the presence of toxic alkaloids (see below).

Systematic Implications of Susceptibility to Other Legume Pests

The Leguminosae may be divided on the basis of susceptibility to Uromyces spp. rusts; the Caesalpinioideae and Mimosoideae are relatively immune to attack, whereas the Papilionoideae represents about 95% of the host species of the family (El-Gazzar, 1981). The phylogenetic distribution of susceptible tribes includes roughly half those listed in Polhill (1981) in a nearly contiguous arc from the Phaseoleae to the Crotalarieae (Fig. 2-1). However,

susceptible members of the Sophoreae are isolated from more advanced tribes by the non-susceptible Tephrosieae, and the Desmodieae and Phaseoleae are isolated from the temperate tribes Trifolieae, Coronilleae, etc. by the non-susceptible Carmichelieae. It may be speculated that the susceptible Sophoreae was at one time linked to the other susceptible tribes through the Tephrosieae, but this bridge no longer exists (or has not been found). The distribution of Uromyces susceptibility is roughly similar to the A. gemmatalis host range, except that the Robineae to Aeschynomeneae New World tribes are immune to Uromyces infection and the Galegeae to Coronilleae temperate tribes are susceptible.

Bruchid beetles are known to feed on several plant families but most (84%) of the hosts are legumes (Johnson 1981). Unlike the Uromyces, the bruchids as a group have a wide host range, feeding on members of all three subfamilies of Leguminosae. Individual species of bruchids, however, are restricted in their host range, most often to a single genus (Johnson 1981). Legume seeds are well protected from herbivores (Janzen 1969) and the bruchid species that utilize select legume genera have adapted to their host defenses (see below).

This suggests that the factors (i.e., nutritional requirements, chemical defenses) determining Uromyces susceptibility and Bruchidae attack are similar to the

factors influencing A. gemmatalis larval host range. Both groups are specific in their host utilization; only select Papilionoideae tribes are susceptible to Uromyces, whereas, the bruchid beetles are very species specific in their host utilization perhaps due to the detoxication of species specific chemical defenses. It may be proposed that Papilionoideae protection from attack by Uromyces or bruchid herbivores may be imparted by alkaloids or phytoalexin isoflavones in the leaves and various seed toxins, non-protein amino acids, alkaloids, lectins, proteinase inhibitors.

Legume Chemosystematics

Apart from morphological and cytological distinctions among the subfamilies of the Leguminosae (Polhill et al. 1981), the Caesalpinioideae and Mimosoideae may be separated from the Papilionoideae by their anti-herbivore defensive strategies. The structurally complex chemical defenses present in the Papilionoideae consist of alkaloids, isoflavonoids, and non-protein amino acids (Polhill et al. 1981; El-Gazzar and El-Fiki 1977). Mimosoideae and Caesalpinioideae chemical defenses most commonly include tannins and terpenoids (Langenheim 1981). Additionally, many of the species of the Mimosoideae and Caesalpinioideae maintain ant-attracting extra floral nectaries that function indirectly as herbivore defenses in place of the

allelochemistry of the Papilionoideae (Polhill et al. 1981). The enzyme systems necessary to produce the complex allelochemicals are considered relatively expensive metabolically (Feeny 1976, Rhoades and Cates 1976). The presumed relatively inexpensive allelochemicals used by the Mimosoideae and Caesalpinioideae, (e.g., tannins and terpenes) suggest that both the synthesis of sophisticated chemical defenses (e.g., alkaloids and non-protein amino acids) and the maintenance of an ant symbiosis may be too metabolically expensive (Janzen 1966). Alternatively, the complex defenses may be unnecessary due to the effective ant defenses or foliar nutritional deficiencies.

Ecological Function of Plant Metabolites

It may be difficult (if not impossible) to unequivocally attribute a specific role to a particular plant metabolite. The ecological roles of many classes of compounds may vary, performing possibly several purposes simultaneously or perhaps at different times in the plant's life cycle (Janzen 1981). As an example, the plant flavonoids are generally believed to function as UV filters, anti-herbivore and anti-pathogen metabolites (Swain 1975). Furthermore, the concentration or presence/absence of the plant compounds may vary among plant populations (Jones 1972, Nass 1972), parts of the same individual (Whitham 1983), seasonally (Feeny 1970) or with the nutritional

status of the plant (Chew and Rodman 1979). Despite several extensive and thorough reviews (e.g., Mears and Mabry 1971, Harborne et al. 1975, Kinghorn and Smolenski 1981, Harborne and Mabry 1982, Harborne 1988a), chemical information regarding specific plant species (and genera) remains fragmentary and incomplete; a few allelochemicals from a relatively small number of legume species have been described (Gomes et al. 1981). Further aggravating the situation, only a few of the A. gemmatilis host or non-host legume species have been chemically described. However, there is a growing body of literature that suggests many plant-derived allelochemicals have a profound influence on insect herbivores. The chemical participants include many classes of compounds among them are the alkaloids, flavonoids, non-protein amino acids, and terpenoids (Harborne 1982), although rarely is a plant species simultaneously protected by several of these chemical classes (Harborne 1982).

Chemical Defenses of the Leguminosae

The vast majority of legume allelochemistry information describes compounds in the species of greatest economic importance (e.g., Glycine, Phaseolus, and Pisum). Frequently, the information is used to describe taxonomic affiliations (chemotaxonomy) within the family (Harborne et al. 1971, Polhill and Raven 1981). This review compiles

information on alkaloids (Willaman and Schubert 1961, Willaman and Li 1970, Mears and Mabry 1971, Smolenski and Kinghorn 1981, Kinghorn and Smolenski 1981), flavonoids (Gripenberg 1962, Hattori 1962, Seshadri 1962, Harborne 1971a, Ingham 1983, Hrazdina 1982, Wollenweber 1982, Harborne and Williams 1982, Bohm 1982, Chopin et al. 1982, Dewick 1982, Harborne and Grayer 1988, Chopin and Dellamonica 1988, Dewick 1988, Wollenweber and Jay 1988, Harborne and Williams 1988, Bohm 1988), terpenoids (Harborne 1971b, Langenheime 1981) and non-protein amino acids (Bell 1971, 1981, Rosenthal 1982) for those legume species represented in laboratory feeding trials (Table 2-1). Only references that pertain to the compounds identified from foliage (or in some cases, 'aerial parts') are included. When complete information is provided by a reviewer, space is saved by citing the review article; however, where the source of the plant material (i.e., plant part) listed in the review article is unknown, the specific reference is cited if it meets the above criteria.

Alkaloids

Alkaloids, heterocyclic nitrogenous compounds, are restricted in their taxonomic distribution to one-seventh to one-third of the families of flowering plants, the majority of which are dicotyledonous (Robinson 1979). With the exclusion of the orders Magnoliales and Ranales which

contain many alkaloid bearing species, the Leguminosae contain (as of ca. 1974) about the same percentage of alkaloid-producing species (41% of 1643 species surveyed) as other dicot families (40% of 4432 species surveyed) (Levin 1976). The distribution of alkaloids within the family is strongly biased toward the Papilionoideae where only 2 of the 15 alkaloid classes analyzed (phenylalanine and bicyclic and tricyclic tryptophane-derived alkaloids) occur in the Casaelpinioideae and Mimosoideae (El-Gazzar and El-Fiki 1977). Nearly 100 compounds have been reported from the Caesalpinioideae and Mimosoideae (Smolenski and Kinghorn 1981) while over 350 have been reported from the species of the Papilionideae (Kinghorn and Smolenski 1981).

Although most alkaloids reported from the Leguminosae occur in seeds, a few occurrences of alkaloids in foliage were reported (Table 2-2). Alkaloids represent one of the most consistently toxic classes of compounds to herbivorous insects (Janzen et al. 1977). Classes of alkaloids reported in foliage of Leguminosae species included in Table 2-1 include erythrina alkaloids from the Erythrina spp., pyrrolizidine alkaloids (e.g., monocrotaline) from Crotalaria spp. and the quinolizidine alkaloids from Lupinus spp. Only the alkaloid classes represented in the species list (Table 2-1) are represented here. For a description of the alkaloid classes see Robinson (1979).

Table 2-2. Literature review of the legume foliar chemistry potentially associated with *A. gemmatalis* adult and larval host selection and performance. Taxa arranged phylogenetically after Polhill and Raven (1981).

Scientific name	Class ⁴	Chemistry	Reference
Caesalpinioideae			
<u>Parkinsonia aculeata</u> L.	FLAV:	parkinsonin-A & B, epiorientin	Bhatia <u>et al.</u> (1966)
<u>Senna occidentalis</u> (L.) Link	Link FLAV:	7-rhamnoside 6-hydroxyluteolin 3'-methyl ether <u>Cassia</u> spp. prod. anthra- quinones widely (e.g. kaempferin, kaempferol, isorhamnetin trigonelline, L-stachydrine choline, betaine	Harborne & Williams (1982) Harborne (1971b) Seshadri 1962)
Mimosoideae	ALK:		Ghosal <u>et al.</u> (1970)
<u>Albizia julibrissin</u> Durazz.	FLAV: ALK:	undetermined anthocyanin phenethylamine 5-hydroxytryptamine, noradrenaline	Nozzolillo (1973) William and Schubert (1961) Applewhite (1973)

⁴ FLAV.=Flavonoid; ALK=Alkaloid; NPAA=Non-protein amino acid; STER=Sterol;
PHEN=simple phenolic; and CYN=Cyanogenic.

Table 2-2--continued.

Scientific name	Class	Chemistry	Reference
<u>Papilionoideae</u>			
<u>Robinia pseudoacacia</u> L.	FLAV:	acacilin, acacetin	Hattori (1962) Gripenberg (1962)
<u>Indigofera hirsuta</u> L.	FLAV:	3,5-digalactoside kaempferol	Harborne & Williams (1988)
	ALK:	unnamed alkaloid	Willaman and Schubert (1961) Domínguez <u>et al.</u> (1978) Ingham (1981)
<u>Indigofera</u>	FLAV:	louisfieserone A,	
<u>suffruticosa</u> Mill.	STER:	pinitol, β -sitosterol	
<u>Desmodium</u>		<u>Desmodium</u> spp. prod.	
<u>floridanum</u> Chapm.	FLAV:	kievitone	
<u>Lespedeza striata</u> (Thunb.) Hok. & Arn.			
<u>Lespedeza</u>		<u>Lespedeza</u> spp. prod.	Ingham (1981)
<u>hirta</u> (L.) Hornem.	FLAV:	kievitone, cristacarpin	
<u>Erythrina herbacea</u> L.	ALK:	unnamed alkaloid	Willaman and Schubert (1961) Ingham (1983)
<u>Mucuna deeringiana</u> (Bort.) Merrill	FLAV:	genistein, 2'-hydroxygenistein, dalbergioidin, isoferreirin, cajanol, kievitone, medicarpin, mackiaian	
<u>Pachyrhizus</u>	FLAV:	demethylmedicarpin, neodunol	Ingham (1983)
<u>erosus</u> (L.) Urban	ALK:	unnamed alkaloids	Willaman and Schubert (1961)

Table 2-2--continued.

[illegible]

Table 2-2--continued.

Scientific name	Class	Chemistry	Reference
	ALK: STER: SAP:	kaempferol and quercetin 3-(2 ⁶ - rhamnosylgentiobioside) trigonelline pinitol saponin	Arnoff (1956) Dreyer et al. (1979) Birk & Peri (1980) Langenheim (1981) Markham & Ingham (1980)
<u>Centrosema</u> <u>virginianum</u> (L.) Benth. <u>Vigna</u> <u>unguiculata</u> (L.) Walp.	FLAV: Benth. FLAV:	tectorigenin, formononetin	
	FLAV:	daidzein, kievitone, medicarpin, phaseollin, phaseollidin, 2'-O-methylphaseollidiniso flavan, coumestrol, vignafuran, cyanidin	Ingham (1983)
	ALK:	unnamed alkaloid	Nozzolillo & McNeill (1985) Willaman and Schubert (1961)
<u>Phaseolus lunatus</u> L.	FLAV:	kievitone, malvidin	Ingham (1983) Nozzolillo & McNeill (1985)
<u>Phaseolus vulgaris</u> L.	FLAV:	kievitone, phaseollidin, phaseollin, 2'-O-methylphaseollidiniso flavan, phaseollinisoflavan, coumestrol, 2'-hydroxygenistein,	Ingham (1983) Dewick (1988)

Table 2-2--continued.

Scientific name	Class	Chemistry	Reference
	ALK:	quercetin-3-glucuronide acetylcholine,	Harborne (1971a) Hartmann & Kilbinger (1974)
		malvidin	Nozzolillo & McNeill (1985)
	SAP: FLAV:	querciturone saponins	Hattori (1962)
<u>Caianus</u>		formononetin, genistein,	Langenheim (1981)
<u>cajan</u> (L.) Druce		2'-hydroxygenistein, pinostrobin, (pos.) longi- styrene A, E, cajanol, cajanin, iso- prenylated genistein	Ingham (1981) Cooksey <u>et al.</u> (1982) Dahiya (1987)
<u>Rhynchosia</u>			
<u>minima</u> (L.) DC.	FLAV:	isovitexin, isoorientin, vicenin-2, vicenin-3	Besson <u>et al.</u> (1977)
<u>Arachis hypogaea</u> L.	FLAV:	medicarpin	Dewick (1988)
	NPA:	4-methyleneglutamine	Done & Fowden (1952)
<u>Astragalus</u>		4-methy 4-hydroxyglutamic acid	
<u>villosus</u> Michx.			
<u>Vicia angustifolia</u> L.	FLAV:	Several spp. prod. pisatin, orobol, medicarpin, maackiain, variabilin, lathycarpin variabilin	Ingham (1981)
<u>Lathyrus</u> sp.			Dewick (1988) Ingham (1983)
<u>Lens culinaris</u> Medic.	FLAV:		
<u>Pisum sativum</u> L.	FLAV:	formononetin, pseudobaptigenin, afroformosin, maackiain, 2-methoxy-	Ingham (1983)

Table 2-2--continued.

Scientific name	Class	Chemistry	Reference
		medicarpin, 2-methoxyhomopterocarpin, pisatin, anhydrosisatin, pseudobaptigenin, kaempferol, quercetin, obtustylene, xenognosin, 4,4'-dihydroxy-2'-methoxychalcone, kaempferol-3-triglucoside, quercetin-3-triglucoside acetylcholine	Dewick (1988) Harborne (1971a) Carlson & Dolphin (1982) Galston (1969)
	ALK:	5-hydroxytryptamine, trigonelline	Hartmann & Kilbinger (1974)
	NPPA:	tyramine, putresine, β -(2,6-dihydroxypyrimidin-1-yl)-alanine, homoserine, O-acetylhomoserine, N-(indole-3-acetyl)aspartic acid	Applewhite (1973) Leete <u>et al.</u> (1955) Miettinen (1955) Brown & Mangat (1969) Grobelaar & Steward (1969) Andrae & Good (1955) Ingham (1983)
<u>Cicer arietinum</u> L.	FLAV:	formononetin, cuneatin, biochanin A, pratensein, medicarpin, maackiain, formononetin 7-O-glucoside, biochanin A -7-O-glucoside, isoliquiritigenin, 3,7,4'-trihydroxyflavanone, isoliquiritigenin-4'-glucoside, daidzein, garbanzol medicarpin,	Harborne (1971a) Wong <u>et al.</u> (1965) Ingham (1977)
<u>Melilotus alba</u> Desr.	FLAV:		

Table 2-2--continued.

Scientific name	Class	Chemistry	Reference
		melilotocarpan A, B, C, D, E, 3-galactosyl(1-6)glucoside-7-di- rhamnoside	Dewick (1988) Harborne & Williams (1988)
	PHEN:	O-coumaric acid, melilotic acid, coumarin	Harborne (1971b)
<u>Medicago lupulina</u> L.			
<u>Medicago sativa</u> L.	FLAV:	daidzein, formononetin, genistein, biochanin A, sativanone, medicarpin, vestitol, coumestrol, 9-O-methylcoumestrol, lucernol, medicagol, trifoliol, 8-methoxycoumestrol, sativol, wairiol, daidzein-7-O-glucoside, formononetin-7-O-glucoside, coumestrol-O-glucoside, 7,5'-dihydroxy- & 7,3',4'- trihydroxyflavone, tricin	Ingham (1983)
	SAP:	soyasapogenols A to E, medicogenic & lucernic acids (as complex glycosides), citrulline stachydrin homostachydrin	Harborne (1971a) Gripenberg (1962) Harborne (1971b)
	NPAA:		Kolousek (1956)
	ALK:		Steenbock (1918)
	ALK:		Willaman and Schubert (1961)
<u>Trifolium repens</u> L.	FLAV:	daidzein, formononetin, 2'-Hydroxy-formononetin, genistein, vestitone, medicarpin, coumestrol, 9-O-methylcoumestrol,	Ingham (1983)

Table 2-2--continued.

Scientific name	Class	Chemistry	Reference
		repensol, trifoliolol, vestitone, demethylmedicarpin 7,4'-dihydroxy- & 7,3',4'- trihydroxyflavone, isoquercetrin soyasapogenols A, B and C as complex glycosides), linamarin, lotaustrolin monocrotaline	Dewick (1988) Harborne (1971a) Hattori (1962) Harborne (1971b) Harborne (1971b) Robins <u>et al.</u> (1974) Culvenor & Smith (1957a) Robins <u>et al.</u> (1974) Culvenor & Smith (1957b)
<u>Crotalaria retusa</u> L.	ALK:	retusine, retusamine monocrotaline spectabiline	Markham <u>et al.</u> (1970)
<u>Crotalaria spectabilis</u> Roth	ALK:	genistein, biochanin A, biochanin A 7-O-glucoside, orbol, orbol 7-O-glucoside, orbol 7-O- rhamnoglucoside, formononetin, formononetin 7-O-glucoside, pseudobaptigenin, pseudobaptigenin 7-O-rhamnoglucoside kaempferol, kaempferol 3-O-glucoside, kaempferol quercetin, quercetin 3-O-glucoside, quercetin 3-O-	Harborne (1971a)
<u>Baptisia lactea</u> (Raf.) Thiret	FLAV:		

Table 2-2--continued.

Scientific name	Class	Chemistry	Reference
	ALK:	rhamnoglucoside, 4'-7-dihydroxy-flavonol, 3-O-glucoside, 3',4', 6 7-trihydroxyflavonol 3-O-glucoside, (4',7-dihydroxy-flavonol 7-O-glucoside, 3',4',7-trihydroxyflavonol 7-O-glucoside, 3',4',7-trihydroxy-flavonol 7-O-rhamnoglucoside) cytosine, methylcytosine, anagyrine, d-sparteine (pachycarpine)	Cranmer & Mabry (1966) Kupchan & Dahle (1961)
<u>Lupinus villosus</u> Willd.			
	FLAV:	luteone (fnd in 11 <u>Lupinus</u> spp.) <u>Lupinus</u> spp. prod kaempferol, quercetin <u>L.</u> spp. prod. apigenin, apigenin 7-O-glucoside, luteolin, luteolin -7-O-glucoside, vitexin, vitexin acyl I, orientin acyl I, orientin acyl II. <u>Lupinus</u> spp. ca. half the spp. tested prod. luteolin, apigenin, quercetin, kaempferol <u>Lupinus</u> spp. prod. sparteine, lupanine, epilupanine, lupinine	Ingham (1983) Harborne (1971a) Nicholls & Bohm (1983) Harborne (1969) Willaman and Schubert (1961)
	ALK:		

Table 2-2--continued.

Scientific name	Class	Chemistry	Reference
		hydroxylupanine, sparteine unnamed alkaloid	

Pyrrolizidine Alkaloids

The pyrrolizidine alkaloids reported from the plant species included here were monocrotaline, retusine and retusamine from C. retusa and monocrotaline and spectabiline from C. spectabilis. No reports of foliar alkaloids were found for C. lanceolata. The compilation of feeding trial information indicates that, no A. gemmatilis larvae survived on C. spectabilis, whereas 40% survived on C. retusa. Neonate larvae (40%) apparently survived on C. retusa foliage until late instars (Plagens unpubl.) yet only a slight amount of feeding was observed on C. spectabilis (Cohen unpubl., Plagens unpubl.).

Pyrrolizidine alkaloids are sequestered by herbivorous larvae (e.g., Utetheisa) and metabolized for an adult courtship pheromone (Connor et al. 1981), for the morphogenesis of scent glands (Schneider et al. 1982) and for defense against spider predators (Brown 1984). Their antiherbivore activity may be due to their bitter taste (Glendinning 1989) and thus, they may be repellent to non-adapted herbivorous species. It is difficult to distinguish between toxicity and repellency with these mortality data (Table 2-1), yet I speculate that the foliar pyrrolizidine alkaloids retusine and retusamine present in C. retusa may be only moderately toxic or repellent whereas spectabiline,

present in C. spectabilis, may be very toxic to A. gemmatalis larvae. As monocrotaline was present in both Crotalaria spp. it is probably not responsible for the mortality of A. gemmatalis larvae.

Quinolizidine Alkaloids

Species within the Genisteae and Thermopsidae (e.g., Lupinus spp., Baptisia spp.) are distinguished from most other Papilionoideae tribes by the occurrence of quinolizidine alkaloids (Polhill 1981). Examples of these alkaloids include (among many quinolizidine alkaloids) epilupinine, hydroxylupanine, lupanine, lupinine, and sparteine from the foliage of Lupinus spp. and sparteine from Baptisia lactea foliage (Table 2-2).

The quinolizidine alkaloids (if not all allelochemicals) may be quite species-specific in their activity toward herbivorous species. High concentrations of quinolizidine alkaloids (i.e., 17-oxosparteine, sparteine, 12,13-dehydrosparteine and lupanine) impart aphid (Aphis cytisorum Hartig) resistance in Cytisus and Lupinus (Wink et al. 1982). Sparteine was also toxic to the bruchid beetle Callosobruchus maculatus (Fabr.) (Janzen et al. 1977) while the same compound is a repellent of the cabbage butterfly Pieris brassicae (L.) (Schoonhoven 1973). The grasshopper, Melanoplus bivittatus (Say), is deterred from feeding on lupinine containing plants (Harley and Thorsteinson 1967).

However, other species may be tolerant of alkaloids or use them as cues for host finding. For example, sparteine from Lupinus spp. and other legume species is not toxic to the aphid Aphis rumicis L. (Tattersfield et al. 1926) yet is a feeding stimulant of the aphids Acyrtosiphon spartii (Koch.) on broom (Smith 1966). In lupines, sparteine comprises one component of a complex of alkaloids that protect against the flower-feeding Glaucopsyche lygdamus Doubleday (Dolinger et al. 1973). These workers also found that several alkaloids occurred simultaneously in lupines and collectively protected the plant as they were in high concentrations but individually the compounds occurred at low levels. The authors speculated that this variability imparted more permanent resistance in the plant as the herbivores would not be likely to metabolize all defensive compounds. Additionally, relatively low concentrations (0.6% dry weight) of lupinine and sparteine incorporated into the artificial diet of the armyworm Spodoptera eridania (Cramer) reduced survival and larval growth (Johnson and Bentley 1988). However, the influence of these alkaloids on the A. gemmatilis host range is uncertain as only two potentially quinolizidine-bearing species were tested (i.e., Lupinus villosus and Baptisia lactea) resulting in 40 and 70% larval survival, respectively.

Erythrina Alkaloids

With regard to the erythrina alkaloids, only an unidentified alkaloid (possibly erysotrine, or α -erythroidine as reported from the foliage of other Erythrina spp., Hargreaves et al. 1974) has been reported from Erythrina herbacea foliage. Additionally, the seeds of E. herbacea contain hypaphorine, erysodine, erysopine glucoerysodine (Mears and Mabry 1971, Hargreaves et al. 1974). The erythrina alkaloids are known to block acetylcholine receptors (Robinson 1979) but as no other chemical information is known for this local species, more work is required to investigate the relationship between the low survival (0%) of A. gemmatalis larvae and E. herbacea allelochemistry.

Miscellaneous Ungrouped or Protoalkaloids

Several other alkaloids and protoalkaloids (non-heterocyclic nitrogen compounds) were reported from plants tested for A. gemmatalis host utilization. These plant species include the non-hosts, Senna occidentalis (0% survival), Albizia julibrissin (0% survival), P. vulgaris (10-27% survival), and the hosts Indigofera hirsuta (87-100% survival), Pachyrhizus erosus (\pm field host), G. max (49-100% survival), P. sativum (40-70% survival), and M. sativa (field host). No clear relationships are apparent from the alkaloid compounds reported and the host status data.

Because several species of alkaloid-bearing papilionoids in the genera Erythrina and Crotalaria did not serve as laboratory hosts of A. gemmatalis (Table 2-1), these alkaloids may be important in determining the host range extension of this herbivore. Collaborative data (Slansky and Wheeler unpubl. data) suggest that A. gemmatalis larvae may be sensitive to alkaloids in general, as the larvae suffered 100% mortality when the purine alkaloid caffeine was incorporated in artificial diet at between 0.1 and 0.5% fresh weight.

A few alkaloids have been reported in the Caesalpinioideae (e.g., cassinine in the Cassia s.l., Mulchandani and Hassarajani 1977) but far more Papilionoideae species produce alkaloids and far more alkaloids are produced by the members of this subfamily.

Non-protein Amino Acids

Non-protein amino acids have been reported primarily from the Leguminosae (Bell 1981) and may be found in all plant tissues but generally are concentrated in the seeds (Rosenthal and Bell 1979). Over 80 non-protein amino acids are known from the Leguminosae; among them, canavanine has been surveyed extensively and has been found only in the Papilionoideae (Turner and Harborne 1967). Although extensive surveys of the remaining compounds have not been conducted, they may be distributed widely in all subfamilies

(see below). The toxicity is generally due to incorporation of non-protein amino acids into proteins and their subsequent failure to function normally (Pines et al. 1981).

Several non-protein amino acids incorporated into artificial seeds were toxic to larvae of the seed-eating bruchid C. maculatus (Janzen et al. 1977) and inhibited feeding in Locusta (Navon and Bernays 1978). The non-protein amino acid L-canavanine was toxic to the tobacco hornworm, Manduca sexta (L.) (Dahlman and Rosenthal 1976). The non-protein amino acid, β -cyanoalanine, disrupts the water balance of Locusta migratoria L. (Schlesinger et al. 1976). However, bruchid beetles (Caryedes basiliensis), adapted to feed on seeds rich in L-canavanine, may use the toxic metabolite as a source of nitrogen (Rosenthal et al. 1982).

Although much information is available on seed non-protein amino acids, very little could be found on their foliar levels because either they rarely occur in foliage or foliar non-protein amino acids have yet to be thoroughly investigated. However, I did find literature citations describing non-protein amino acids (Table 2-2) occurring in Arachis hypogaea, P. sativum, M. sativa, all considered A. gemmatalis hosts (Table 2-1).

Also included here are the plants that sequester selenium from soils and incorporate it in amino acids. These frequently occur in the legume genus Astragalus (among

others) (Rosenthal and Bell 1979), which often grow where selenium is abundant in soils (Shrift 1969). Although these accumulator plants are toxic to humans and livestock (Shrift 1969), few examples of insect toxicity were found. Bruchids and seed chalcids, adapted to feeding on high selenium-containing Astragalus seed pods, were very tolerant of levels toxic to various mammals (Trelease and Trelease 1937). However, the foliage of selenium-accumulating Astragalus sp. was toxic to aphids and various mite adults and eggs (Gnadinger 1933, Hurd-Karrer and Poos 1933).

Only a single species of this genus, A. villosus, a non-accumulator (Barneby 1964), has been tested as a potential host plant of A. gemmatalis with 100% larval survival (Table 2-1). Thus, the information reviewed suggests that non-protein amino acids have little influence on the host range of A. gemmatalis larvae.

Flavonoids

The flavonoids comprise more than 4000 unique chemical structures (Harborne 1988a) and are widely distributed throughout the angiosperms (Swain 1975). This estimate may represent only a fraction of the flavonoids described, considering the small percentage of the plant species thoroughly investigated (Harborne 1988b). Flavonoids frequently serve as either herbivore attractants or deterrents depending upon the compound and/or herbivore

species (Harborne 1988b). Flavonoids may be involved in regulation of rhizobium root nodulation, or protection from fungal pathogens (Harborne 1988b), nematodes (Kaplan et al. 1980) or insect herbivores (Hart et al. 1983).

Among the many classes of flavonoids (Harborne 1988a), I will discuss only those that have been shown to influence herbivore performance, namely the flavones, flavonols, flavonoid glycosides, isoflavones and anthocyanins.

Flavone and Flavonols

Flavones and flavonols are generally localized in surface foliar tissues or structures (e.g., glandular trichomes) (Wollenweber and Dietz 1980) and are associated with lipophilic substances (e.g., terpenoids and waxes) (Wollenweber 1982). These natural products are not widely known from members of the Leguminosae (Wollenweber and Jay 1988).

Subfamily differences in flavonoid allelochemistry.

Despite the occurrence of flavonoids in all tribes of the family (and all flowering plants examined) some groups of compounds predominate in select legume subfamilies. The common flavonols myricetin, quercetin and kaempferol are almost exclusively restricted to the arborescent Caesalpinioideae and Mimosoideae (Harborne 1971a). The tannins of the leucoanthocyanidin group (proanthocyanidins) are widely distributed in these two subfamilies, whereas

they occur in only a few woody Papilionoideae species (Haslam 1982). On the other hand, two common flavones, luteolin and apigenin, have been recorded only from the Papilionoideae (El-Gazzar & El-Fiki 1977).

This raises the possibility that the mentioned flavonols and tannins are toxic or deterrent, thereby limiting the utilization of the Caesalpinioideae and Mimosoideae, whereas the Papilionoideae flavones attract or are otherwise innocuous to A. gemmatalis larvae. Kaempferol was reported from many species of the non-host genus Cassia; however, kaempferol and quercetin glycosides were reported from foliage of the Papilionoideae species G. max, P. sativum, P. vulgaris and Baptisia lactea (Table 2-2). Of this group, only P. vulgaris is regarded as a non-host (Table 2-1). Additionally, kaempferol and quercetin have been reported from many Lupinus spp. (Table 2-2), and L. villosus is a non-host of A. gemmatalis. Furthermore, both the flavones luteolin and apigenin commonly occur in Lupinus spp. Thus, as the flavonols were reported from both the Caesalpinioideae and Papilionoideae and from host and non-hosts they apparently do not have an influence, or do not occur at concentrations that influence the A. gemmatalis host range.

A survey of the flavonoids of 73 species of Lupinus from western North America revealed some generalities of the genus. Although our local species, L. villosus was not

included, representative flavonoids of the genus may include the flavones (and many glucosidic forms) apigenin, acacetin (also present in the A. gemmatalis potential host R. pseudoacacia), luteolin, chrysoeriol, and the flavonols kaempferol, quercetin, vitexin and orientin (Nicholls and Bohm 1983). The relatively low percent survival (40%) observed when A. gemmatalis larvae were reared on L. villosus (Table 2-1), suggests that one or more of these flavonoids may be toxic or repellent under these circumstances. Furthermore, the lupines contain many active alkaloids (see above) that may have contributed to the observed larval mortality.

Insect herbivore bioassays of several of these compounds revealed considerable activity. The flavones vitexin, luteolin and the flavonols quercetin, quercitrin, rutin, morin, myricitrin and tricin extracted from wheat, deterred feeding of one or both aphid species Schizaphis graminum (Rondani) and Myzus persicae (Sulzer) (Dreyer and Jones 1981). Quercetin, catechin (a flavan-3-ol), and naringenin (a flavanone), when incorporated into artificial diet, were all toxic to the aphid S. graminum (Todd et al. 1971). Larval growth of Heliothis zea (Boddie), H. virescens (Fabr.) and Pectinophora gossypiella (Saunders) was reduced when their artificial diet contained the flavonol quercetin or two of its glycosides, quercetrin or rutin (Shaver and Lukefahr 1969, Elliger et al. 1981). In

contrast, rutin added to the artificial diet of the cricket Acheta domesticus (L.) accelerated growth (McFarlane and Distler 1982, Neville and Luckey, 1971).

The glycosides of myricetin and quercetin from Rhus spp. and Schinus stimulated feeding by the flea beetles Blepharida spp. (Furth and Young 1988). Other flavonoid glycosides have stimulated feeding in several herbivore species: luteolin-7-glucoside, isolated from Salix gracilistyla foliage, stimulated feeding of the leaf beetles Chrysomela vigintipunctata costella (Marseul) and Lochmaea capreae cribrata Solosky (Matsuda and Matsuo 1985), and the beetle Agasicles sp. uses 7- α -L-rhamnosyl-6-methoxyluteolin as a feeding attractant to its host plant alligatorweed (Alternanthera phylloxeroides) (Zielske et al. 1972). The six flavonol glycosides of kaempferol and quercetin, isolated from soybean leaves (Buttery and Buzzell 1975), may similarly stimulate feeding in A. gemmatalis larvae.

Flavonoids utilized as ovipositional stimulants have been reported for two swallowtail butterflies, Papilio spp., both on Citrus. The flavanone glycosides naringin and hesperidin from sour orange, Citrus natsudaoidai, were synergistic in their activity, only active when combined or when included in an unidentified mixture (Honda 1986). The extracts of fresh leaves of C. unshiu containing 6,8-di-C- β -D-glucopyranosylapigenin were also synergistic with other compounds in an unidentified mixture (Ohsugi et al. 1985).

The aggregations of A. gemmatalis adult males on sweep nets repeatedly swept through soybean foliage suggest a similar stimulant (Gregory 1986). Perhaps A. gemmatalis males locate suitable habitats by flavonoids released when foliage is injured by herbivores or sweep nets. However, the attraction of adult Lepidoptera to salts (e.g., puddling) is well documented (Arms et al. 1974) and may also explain these aggregations.

Isoflavones

The isoflavones constitute one of the most active classes of legume compounds. Isoflavones are synthesized de novo (phytoalexins) when elicited by topical application of cupric chloride (Burden and Bailey 1975), irradiation with UV light (Hart et al. 1983) or pathogen infusions (Keen et al. 1971, Ingham et al. 1981). Among their many activities, these compounds are oestrogenic in livestock, antifungal, antibacterial (Ingham 1983) and deter herbivore feeding (Hart et al. 1983). Additionally, their toxicity to insects is well known, as demonstrated by the insecticide rotenone, extracted from Derris roots (Krukoff and Smith 1937). The fractions containing the isoflavones phaseol and afrormosin (also present in P. sativum and Centrosema spp. foliage) extracted from resistant (PI-227687) soybean foliage were highly toxic (71-98% mortality) to the soybean looper Pseudoplusia includens (Walker) (Caballero et al. 1986).

Many other examples of isoflavones are distributed widely in the Papilionoideae (Table 2-2, e.g., citations by Dewick 1982, Ingham 1983, Dewick 1988) but their activity toward insects is largely unexamined. However, a few surveys have been conducted primarily with the root-boring scarab beetle, Costelytra zealandica White, with which feeding stimulation or repellency was assessed (Table 2-3). The results suggest that both A. gemmatalis non-host and host species contain isoflavones that deter scarab feeding. Some of these repellent isoflavones that occur in A. gemmatalis non-hosts include phaseollin, phaseollidin, genistein, kievitone, formononetin and coumestrol. Commercial coumestrol, however, had no impact on P. includens when incorporated alone in artificial diet, however it may be but one component of herbivore resistance in G. max (Smith 1985). The picture is far from clear regarding the impact of the majority of the isoflavones in A. gemmatalis host or non-host species. However, the greatest activity (towards P. includens) occurs in G. max foliage, namely the isoflavones phaseol and afrormosin.

Anthocyanins

The anthocyanins are generally regarded as plant pigments located in flowers fruits, leaves and storage organs (Harborne and Grayer 1988). However, anthocyanins are also produced in response to stress and form the red

Table 2-3. The influence of various isoflavones on the feeding of several insect herbivores ¹.

Isoflavone	Source (Table 2-2)	Feeding ²
phaseollin	<u>V. unguiculata</u> , <u>P. vulgaris</u> ,	- ³
phaseollidin	<u>V. unguiculata</u> , <u>P. vulgaris</u> ,	- ³
vestitol	<u>M. sativa</u>	- ³
2'-hydroxy-		
formononetin	<u>M. deeringiana</u> , <u>T. repens</u> ,	- ³
2'-hydroxy-		
genistein	<u>P. vulgaris</u> , <u>C. cajanus</u> , <u>T. repens</u> ,	- ³
medicarpin	<u>M. deeringiana</u> , <u>V. unguiculata</u> ,	- ³
	<u>A. hypogaeae</u> , <u>Lathyrus</u> spp.,	
	<u>C. arietinum</u> , <u>M. alba</u> , <u>M. sativa</u> ,	
	<u>T. repens</u> ,	
maackiain	<u>M. deeringiana</u> , <u>Lathyrus</u> spp.,	- ³
	<u>P. sativum</u> , <u>C. arietinum</u> ,	
pisatin	<u>Lathyrus</u> spp. <u>P. sativum</u> ,	- ³
kievitone	<u>Desmodium</u> spp., <u>Lespedeza</u> spp.,	- ³
	<u>M. deeringiana</u> , <u>V. unguiculata</u> ,	
	<u>P. lunatus</u> , <u>P. vulgaris</u>	
luteone	<u>Lupinus</u> spp.	- ³
genistein	<u>M. deeringiana</u> , <u>G. max</u> , <u>B. lactea</u> ,	
	<u>M. sativa</u> , <u>C. cajan</u> , <u>B. lactea</u>	NS ³
biochanin A	<u>C. arietinum</u> , <u>M. sativa</u> , <u>B. lactea</u>	NS ³

Isoflavone	Source (Table 2-2)	Feeding
formononetin	<u>C. virginianum</u> , <u>C. cajanus</u> , <u>P. sativum</u> , <u>C. arietinum</u> , <u>M. sativa</u> , <u>T. repens</u> , <u>B. lactea</u>	NS ³
coumestrol	<u>G. max</u> , <u>V. unguiculata</u> , <u>P. vulgaris</u> , <u>T. repens</u> , <u>M. sativa</u>	NS ³ & ⁴

¹. Sources: Russell et al. (1978), Lane et al. (1985),
Dreyer et al. (1987), Lane et al. (1987).

². Feeding deterrent (-), or no change in feeding (NS)

³. Feeding tests conducted on Costelytra zealandica; or

⁴. Aphid feeding test.

color of autumn leaves and the halo surrounding the site of pathogen infection (Hrazdina 1982). Although not implicated, the poor performance (low pupal biomass) of A. gemmatalis larvae fed senescent soybean foliage (Moscardi et al. 1981) may be attributed to the accumulation of anthocyanins; however, the impact of reduced nutritional leaf quality (e.g., nitrogen, Egli et al. 1978) during this soybean plant phenophase can not be ruled out. Furthermore, anthocyanins have yet to be reported from mature or even senescent soybean foliage. However, the anthocyanin malvidin has been reported from the hypocotyl and stem of G. max seedlings (Nozzolillo 1973) and is possibly translocated to senescent foliage.

Further evidence for the toxicity of anthocyanins occurs in the non-host Albizia julibrissin, found to contain an unidentified anthocyanin in foliage. Additionally, anthocyanins were reported from the non-hosts Lens culinaris (stems; delphinidin), Lupinus sp. (hypocotyl, cotyledon, petiole; cyanidin), P. vulgaris (hypocotyl and stem; malvidin), P. lunatus (hypocotyl and stem; malvidin) and Sesbania punicea (hypocotyl and stem; cyanidin and delphinidin), Wisteria frutescens (stem; unidentified) and from the hosts Medicago sativa (hypocotyl and stem; unidentified), M. lupulina, (hypocotyls; cyanidin) Vigna unguiculata (stem, petiole and leaf; cyanidin) and V. luteola (stem, unidentified) (Nozzolillo 1973, Nozzolillo

and McNeill 1985). Because many of these do not occur in the foliage they are not noted in Table 2-2.

Only three studies were found evaluating the effect of anthocyanins on insect herbivores. The cotton leaf anthocyanin cyanidin 3-glucoside inhibits larval growth, possibly by reducing nutrient digestibility, in the cotton pest H. viriscens (Hedin et al. 1983). However, the tomato pest H. zea is not adversely affected by the tomato anthocyanin petanin (Isman and Duffey 1982, Harborne and Grayer 1988). Thus, no clear interpretation of these limited data may be made regarding the influence of anthocyanins on A. gemmatalis survival. The fact that they occur in leaves of the non-host A. julibrissin suggests they may be toxic or repellent. In some circumstances (e.g., foliage senescence) the compounds may accumulate in tissues normally fed upon. However, at this point I can not make a definitive statement regarding their influence on A. gemmatalis host range.

Terpenoids

The terpenoids are widely distributed throughout all living organisms and comprise many biologically important classes of compounds (Mabry and Gill 1979). Examples of this diverse group include monoterpenes, such as the pyrethroids from Chrysanthemum (Compositae) and the resin constituents of coniferous trees (e.g., Pinus ponderosa,

Abies grandis); sesquiterpene lactones frequently associated with leaves (e.g., glandular trichomes); and the triterpenoid cucurbitacins and saponins (Mabry and Gill 1979). Although legumes (Caesalpinoideae) containing foliar terpenoids have been reported to affect the performance of associated herbivores (Langenheim et al. 1986), terpenoids, with the exception of the saponins, have not been reported in the leaves of potential A. gemmatalis host species (Table 2-2).

Saponins occur in the foliage of several forage legumes, including alfalfa and ladino clover (Applebaum and Birk 1979). Alfalfa and soybean saponins may have several modes of action against insect herbivores; they may inhibit the enzymes α -chymotrypsin and cholinesterase in Tribolium midguts (Ishaaya and Birk 1965), they may be toxic due to sterol inhibition (Birk and Peri 1980, Shany et al. 1970) or herbivore consumption may be reduced due to their bitter taste (Applebaum and Birk 1979). Most of the examples of saponin activity have been directed toward the Coleoptera, however, a few examples were found including aphids and stinkbugs.

Saponins from soybean seeds were toxic to the weevils Callosobruchus chinensis L. and Sitophilus oryzae (L.) (Applebaum et al. 1965, Su et al. 1972). Horber (1964, 1972) found non-preference and antibiosis against the white grub (Melolontha vulgaris F.) in high saponin content roots

of resistant alfalfa strains. Pea aphids, Acyrtosiphon pisum (Harris), were the only insect herbivore of 5 tested species to be affected by the high foliar saponin content of alfalfa cultivars imparting resistance (Pedersen et al. 1976). Saponins, when included in an artificial diet, caused 35% mortality and increased developmental time ca. 3-fold in the aphids M. persicae. Additionally, in choice tests, 100% of the aphids (n=40) preferentially fed on an unadulterated diet instead of a saponin treated diet (Schoonhoven and Derksen-Koppers 1976). However, developmental times of nymphs and adults of the southern green stinkbug, Nezara viridula (L.), were not affected by saponin (from Gypsophylla sp., Caryophyllaceae) added to artificial diet (Bowen 1988). Furthermore, saponins apparently function as a feeding attractant to the Mexican bean beetle in P. vulgaris foliage (Nayar and Fraenkel 1963, citing Lippold 1957).

Saponins have been reported from the foliage of four potential legume hosts of A. gemmatilis (G. max, P. vulgaris, M. sativa and T. repens) (Table 2-2). Although there are no larval rearing data on M. sativa (Table 2-1), only P. vulgaris is a non-host (10-27% survival, Table 2-1).

Saponins do not appear to explain the A. gemmatilis host range observed in Table 2-1. Considering that saponins are most active (foaming) at pH's of 4.5-5.0 (Mangan 1958, 1959), their activity may be minimal in lepidopterous larval

midgut where pH's are generally in the range of 8.3-8.7 (Berenbaum 1980). Although the foaming action of saponins is considered to be responsible for bloating in ruminants, the influence of foaming on insect herbivore toxicity is as yet unknown.

Cyanogenic Species

Cyanogenic plants release hydrogen cyanide gas (HCN) when tissues containing enzymes and cyanogenic precursors (e.g., cyanogenic glycosides) are disrupted. Approximately 500 genera in 100 families contain cyanogenic species. The Leguminosae is noted as one of the major sources of cyanogenesis, containing 125 cyanogenic species (Conn 1979). While the toxicity of these compounds to mammals is well documented, little attention has been given to their toxicity toward insects (Conn 1979).

Of the potential host species listed in Table 2-1, only three produce foliar cyanogenic compounds. These include linamarin and lotaustralin from T. repens foliage and phaseolunatin from P. lunatus and P. vulgaris. Rearing data (Table 2-1) suggest that while the two Phaseolus spp. were poor hosts, A. gemmatilis larval survival on T. repens foliage was 81-90%, suggesting this species was a suitable host.

The difference in toxicity among the different cyanogenic species may have many potential causes. Possible

explanations include the relative toxicity of the different cyanogenic glycosides found in the plant species (e.g., linamarin vs phaseolunatin), their susceptibility to detoxication or concentration differences among the host species. Furthermore, populations of T. repens are polymorphic where both cyanogenic and acyanogenic individuals occur (Jones 1972, Nass 1972) and possibly plants used in the feeding trials lacked toxic levels of cyanide.

On the other hand, the cyanogenic glycoside phaseolutin at low concentrations in P. lunatus and P. vulgaris foliage elicited a strong biting response from the Mexican bean beetle, while at high concentrations phaseolutin acted as a feeding deterrent (Nayar and Fraenkel 1963). Similarly, larvae of the southern armyworm, S. eridania, were successfully reared on cyanogenic Lotus corniculatus possibly due to detoxication of the cyanide (Scriber 1978). Furthermore, S. eridania larvae grew as well or better on diets containing cyanide than on diet lacking added cyanide (Brattsten et al 1983). It is not known, however, if any of these cyanogenic glycosides elicit a similar response.

Proteinase Inhibitors

Proteinase inhibitors occur widely in all plant life and may serve several functions including the regulation of proteolytic enzymes and the protection of tissues from

herbivory (Ryan 1979). Knowledge of the taxonomic distribution of this group of chemicals is rather incomplete; the majority of studies surveyed included only seeds from economically important plant species (Ryan 1979).

Accumulation of proteinase inhibitors, mediated by a wound hormone proteinase inhibitor inducing factor (PIIF), occurs when tomato and potato foliage is damaged by the beetle, Leptinotarsa decemlineata (Say) (Green and Ryan 1972). The wound hormone was translocated throughout the plant triggering an immunological response to herbivory. Wound-induced tomato proteinase inhibitors reduced the larval growth rate of the beet armyworm, Spodoptera exigua (Hübner) (Broadway et al. 1986).

Proteinase inhibitors were also very active in the foliage of other legume species to both endogenous inducers and the known tomato inducer (Walker-Simmons and Ryan 1977). The most actively induced species was M. sativa, while other species responded with minor activity to the inducers (L. culinaris, P. vulgaris, P. sativum, and T. repens). No rearing data are available for A. gemmatalis feeding on M. sativa; however Ellisor and Graham (1937) reported collecting larvae in an alfalfa field. Pisum sativum and T. repens are hosts of A. gemmatalis, but, L. culinaris, and P. vulgaris are considered non-hosts (Table 2-1). Thus, the occurrence of proteinase inhibitors in these species does not consistently explain the host range data.

Miscellaneous Defenses

Lectins

I have reviewed several other classes of compounds that have been reported almost exclusively in tissues other than foliage or have not been reported from species included in Table 2-1. These include the lectins, or phytohemagglutinins, that, although they occur in over 600 species of legumes, are reported in highest concentrations in seeds (Toms and Western 1971). Lectins are probably synthesized in leaves and rapidly translocated to seeds perhaps too quickly for detection (Liener 1979). Lectins are toxic to non-adapted species of insect herbivores (Janzen et al. 1976). However none of the plant species listed in Table 2-1 have been reported to contain lectins.

Simple Phenolics

Coumarins, like the cyanogenic compounds, are hydrolyzed by compartmentalized enzymes and substrates following tissue disruption (Haskins and Gorz 1961). Coumarins may act as either insect herbivore attractants or feeding deterrents (Manglitz et al. 1976). Coumarins have been reported from Melilotus alba foliage (Table 2-2), and this species served as an A. gemmatilis host, with 56-96% survival (Table 2-1). Thus, coumarins may not restrict the host range of A. gemmatilis.

In a search for the chemical nature of resistance in soybean foliage to insect herbivores, a higher sterol content was reported from insect resistant foliage compared with susceptible foliage (Tester 1977). However, the direct bioassay of the identified soybean foliar sterols failed to reveal correlations between insect resistance and sterol content or imbalance (Grunwald and Kogan 1981). The soybean foliage sterol pinitol (also present in Indigofera suffruticosa), when incorporated into artificial diet and bioassayed, reduced growth of H. zea larvae (Dreyer et al. 1979) and the mechanism of this reduced growth was revealed as inhibition of consumption and reduced feeding efficiency (ECD, Reese et al. 1982).

Nutritional Limitations

The production of symbiotic nitrifying bacteria (e.g., Rhizobium spp.) in root nodules, is generally restricted to the Caesalpinioideae and Papilionoideae. Root nodules are generally lacking in non-legumes and the Mimosoideae (Corby 1981). Herbivore species specialization on members of the Leguminosae may be due to the intake of greater amounts of root nodule-produced nitrogen present in legume foliage compared with other forage species (e.g., temperate and tropical grasses, Lyttleton 1973). Foliage feeders may be frequently nitrogen-limited; when fed low nitrogen content foliage, they often exhibit reduced feeding efficiencies and

growth rates, and increased consumption rates compared with foliage of higher nitrogen content (Tabashnik and Slansky 1987).

However, nodule-produced nitrogen in soybean (allantoin and allantoic acid) may not be as available to foliage feeding herbivores (e.g., Epilachna varivestis Mulsant) as amino acid nitrogen (Todd et al. 1972, Wilson and Stinner 1984). The availability of nodule-produced nitrogen in other legume species to herbivores is not known. Thus, nodule produced nitrogen does not entirely explain the A. gemmatalis host range including only the Papilionoideae. The nitrogen utilization efficiency of nodule produced versus amino acid nitrogen has yet to be determined for A. gemmatalis larvae.

Physical Mechanisms of Defense

While I have not exhaustively reviewed the physical characteristics that impart legume resistance, published accounts where these mechanisms may be important are listed here. The mode of action of the Desmodium spp. defenses involved hooked trichomes that entangled young caterpillars (Cohen unpubl.) and damaged mandibles and crochets (Plagens unpubl.). Similarly, mortality (13%) due to glandular trichomes was found when the cotton pest H. virescens was reared on the alternate host D. tortuosum (Hallman 1985). Foliage consumption and A. gemmatalis larval weights were

reduced when fed G. max tawny pubescent genotypes (possibly due to anthocyanins) compared with the gray pubescent genotypes under field and laboratory conditions (Lurding 1984).

Conclusions

The evolution of the larval host range of A. gemmatalis within the Papilionoideae may be traced phylogenetically back to the Tephrosieae. Host utilization patterns follow the divergences in Papilionoideae taxa in the Old World and New World tropics and temperate regions. No species of the phylogenetically isolated African Crotalarieae serve as A. gemmatalis larval host plants.

The most active compounds thus far discovered toward A. gemmatalis larvae include the isoflavones afrormosin and phaseol. Additionally, the pyrrolizidine alkaloids present in the Crotalaria spp. may limit exploitation of this genus by A. gemmatalis larvae.

The occurrence of afrormosin and phaseol only in the host species G. max suggests that many other factors may have influenced the compiled data (e.g., concentration, toxicity, detoxication). Considering the degree of evolutionary interactions occurring at the biochemical level that may have occurred between A. gemmatalis and its host plants, the description of presence or absence of compounds may be misleading.

CHAPTER 3
TOXICITY OF NON-INDUCED AND HERBIVORE-INDUCED
EXTRACTABLES FROM SUSCEPTIBLE AND RESISTANT SOYBEAN
FOLIAGE TO NON-ADAPTED AND SOYBEAN-ADAPTED NOCTUID HERBIVORES

Introduction

Virtually all soybean [Glycine max (L.) Merrill] germplasm being developed for resistance to herbivores is derived from the plant introductions PI171451, PI227687 or PI229358 (Herzog et al. unpubl.). Because these genotypes lack acceptable agronomic characteristics, they are used primarily as breeding lines for the development of resistant cultivars (Hartwig and Edwards 1985). The latter two genotypes adversely affect nearly all major soybean herbivores (Smith 1985). The mode of action of these defenses is a combination of antixenosis, where larval feeding is reduced, and antibiosis, where high larval mortality occurs (Reynolds et al. 1984). The chemical mechanisms of soybean foliar resistance have been discussed in Chapter 2.

Increased levels of insect resistance following herbivory, or induced resistance, are correlated with greater amounts of alleged plant defenses (Schultz and Baldwin 1982). Induced resistance reduces subsequent

herbivore performance (Haukioja and Niemelä 1977, Wallner and Walton 1979, Raupp and Denno 1984) and herbivore numbers (Karban and Carey 1984, Karban et al. 1987). Following herbivory, a mobilization and/or de novo synthesis of allelochemicals may systemically protect foliage adjacent to the feeding site, and sometimes throughout the plant, from further herbivory (Schultz and Baldwin 1982, Karban and Carey 1984). Induced resistance in soybean foliage has been reported to reduce herbivore growth and consumption (Hart et al. 1983, Reynolds and Smith 1985, Chiang et al. 1987). This study describes the chemical mechanisms of constitutive and induced resistance found in soybean foliage and determines their impact on non-adapted and adapted soybean herbivores.

Methods and Materials

Experiment 1. Induction of Susceptible and Resistant Soybean

Agronomic practices. Two soybean lines (provided by E. E. Hartwig, USDA Delta Branch Experiment Station, Stoneville, Mississippi) were planted in late July 1986 at the North Florida Research and Education Center (NFREC) at Quincy, Florida. The soybean genotypes consisted of the resistant plant introduction PI229358 and the susceptible commercial variety Bragg. Plots comprising 2 rows, 6.1 m (20 feet) in length with 180 seeds per row, were treated prior to planting with 561.4 kg/ha (500 lbs/acre) 0-10-20

fertilizer and alachlor at 4.7 l/ha (2 qts/acre). All plants were maintained insect-free with 2 applications of methyl parathion 4E at 0.3 l/ha (0.25 pt./acre). The final insecticide application was made 4 weeks prior to bioassays of soybean foliage.

Induction methods. Field collected 5-6th instar velvetbean caterpillar (VBC), Anticarsia gemmatilis Hübner, larvae were placed in individual organdy leaf cages (15 x 25 cm) covering soybean trifoliates. Four larvae were confined within each cage until ca. 50% of the contained foliage was consumed (ca. 24 h). The cages were placed on all alternate leaves of plants (10-15 leaf cages per plant) between the R3-R5 phenophases (Fehr et al. 1971). Following larval damage (48 h), the adjacent undamaged leaves were collected and immediately stored in an ice chest (ca. 10°C) and brought back to the laboratory for feeding trials. Uninfested, but similarly caged leaves from soybean plants similarly treated were included as controls.

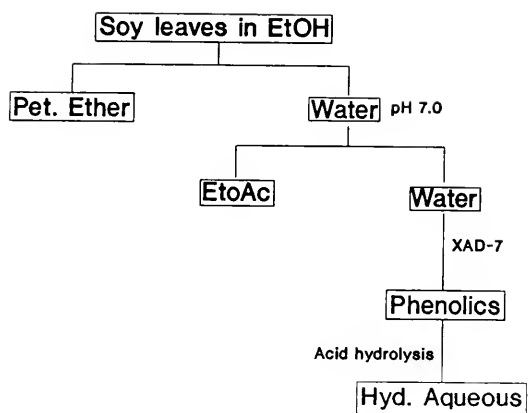
Rearing methods. Larvae (third instar) of the velvetbean caterpillar, the soybean looper (SBL) Pseudoplusia includens (Walker), and green cloverworm (GCW) Plathypena scabra (F.) were field collected at the NFREC from untreated soybean (var. Kirby). Individual larvae were reared in petri dishes (15 x 150 mm) on the leaves of undamaged or damaged soybean plants under standardized environmental conditions (27°C, 50 ±10% RH and 14:10 L:D).

All foliage was presoaked for 30 min. in a 1% bleach (sodium hypochlorite) solution. Leaf petioles were inserted into stoppered water vials to maintain leaf turgidity and replaced once during the first three days of the experiment, and daily thereafter until larval pupation. Larval dry weight (dw) gain and relative growth rates (RGR) were calculated according to a gravimetric method (Waldbauer 1968, Slansky and Scriber 1985). All weights were obtained using an electronic balance (Mettler AC-100, ± 0.1 mg). Similar field collected larvae ($n=20$) were weighed fresh and again after oven drying (60°C for 48 h) to estimate the initial percent dw.

Experiment 2a. Preliminary Extract Methods

Soybean foliage from a commercial variety (Kirby, R-3 plant phenological stage) grown at the NFREC was hand harvested, frozen (-10°C) and extracted with 95% ethanol for 3 days followed by a series of non-polar to polar organic solvents (Fig. 3-1). The ethanol extract was rotoevaporated (60°C) to dryness and the residue partitioned between water and petroleum ether. Following removal of residual petroleum ether, the water fraction was partitioned between ethyl acetate and water. The water fraction was rotoevaporated to remove residual ethyl acetate and passed through an Amberlite XAD-7 non-ionic (Aldrich Co.) column (4.2×29.0 cm) previously washed with 200 ml of deionized

Fig. 3-1. Soybean foliage extract partition scheme.
EtOH=ethanol, Pet. Ether=petroleum ether, EtoAc=ethyl acetate, XAD-7=Amberlite XAD-7 non-ionic resin, Phenolics=phenolic compounds from the water fraction, Hyd. Aqueous=hydrolyzed aqueous fraction.



water. Material adhering to the column was washed with deionized water (200 ml) and eluted with 70% methanol (200 ml). The wash water lacked UV absorbance (265 and 320 nm) and was thus discarded. The methanol eluate was rotoevaporated to dryness and a sample (1 g, dw) was hydrolyzed (to cleave sugars from glycosides) by refluxing for 30 min ca. 60°C followed by extraction with ethyl acetate. All fractions (petroleum ether, ethyl acetate, water extractables (phenolics) and hydrolyzed aqueous extractables (after extraction with ethyl acetate) were rotoevaporated to dryness and refrigerated (10°C).

Diet preparation. Each dry extract fraction was dissolved in methanol, incorporated into a 5% fresh weight (fw) agar-water solution at 1, 2 and 5% (fw) and applied with a fine brush to the upper surfaces of susceptible soybean leaves (var. Kirby). Approximately 2 ml of each treatment solution was applied to individual leaves. A methanol and agar (5% fw) control was also included. All treated leaves (15 replicates) were air dried and petioles were inserted into a water vial and placed in petri dishes (150 x 15 mm).

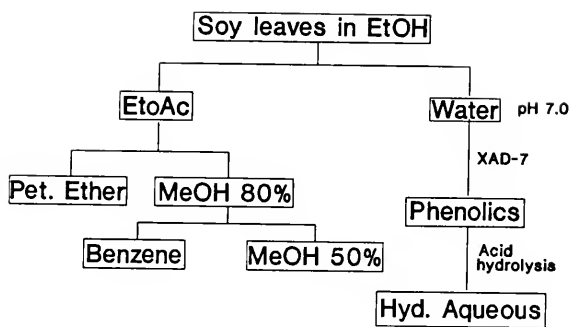
Larval rearing. Field collected fourth instar soybean looper larvae were weighed and reared on treated leaves for 48 h under standard environmental conditions (Experiment 1). Larval percent dw was determined as described above (Experiment 1) and final larval dw and feces production were

recorded. Treatments and blocks were arranged in a randomized complete block experimental design and the data were analyzed by a two-way analysis of variance (ANOVA), where extract fraction and concentration constituted the main effects. Means were separated with a least square means test, maintaining the experimentwise error rate at 5% with the Bonferroni inequality (Sokal and Rohlf 1981), using SAS/PC (SAS Institute, Inc. 1987).

Experiment 2b. Refined Soybean Foliar Extraction

In contrast with the previous procedure that fractionated the water layer, this procedure fractionated the organic solvent extractables (Fig. 3-2) as activity was previously detected in the organic solvent fraction (see Experiment 2a results) and ethyl acetate extractables contained activity (Chiang et al 1987). Soybean foliage (var. Kirby) was collected and stored as described in Experiment 2a. The leaves were extracted for 3 days with ethanol (95%) and the solvent was removed as in Experiment 2a. These ethanol extractables were partitioned between water and ethyl acetate. The ethyl acetate fraction was further partitioned between petroleum ether and 80% methanol. The methanol fraction was partitioned between benzene and 50% methanol. The aqueous extractables were prepared as in Experiment 2a. All fractions were dried and stored as described previously (Experiment 2a).

Fig. 3-2. Soybean foliage extract partition scheme.
EtOH=ethanol, Pet. Ether=petroleum ether, MeOH=methanol,
EtOAc=ethyl acetate, XAD-7=Amberlite XAD-7 non-ionic resin,
Phenolics=phenolic compounds from the water fraction, Hyd.
Aqueous=hydrolyzed aqueous fraction.



Each solvent-free fraction was mixed with cellulose (alphacel, ICN Biochemicals, Inc.) in an excess of acetone, rotoevaporated to dryness. The cellulose was incorporated into an artificial diet at 5% fw (Greene et al. 1976). Treatment diets consisted of fraction concentrations of 0.5 and 1% dw except the methanol fraction due to a shortage of material (0.1 and 0.5% dw). Additionally, the active fraction from Experiment 2a (petroleum ether) was included. A control diet consisted of cellulose (5% fw) and acetone mixed and rotoevaporated before combining with the standard artificial diet. Diet samples (± 500 mg, $n=15$) were weighed fresh, oven dried and reweighed to estimate their initial percent dry matter.

Insects and rearing methods. Third instar velvetbean caterpillar larvae (obtained as eggs from the USDA-ARS Insect Attractants and Basic Biology Laboratory, Gainesville, Florida) were weighed and reared at standard laboratory conditions as previously described (Experiment 1) for 5 d in inverted 30 ml (1 oz) plastic cups lined with a 2 ml layer of Gelcarin HWG (1.5% fw, Marine Colloids, Inc) along the cup top. Larval consumption, growth, and feeding efficiencies were calculated according to a gravimetric technique (Waldbauer 1968, Slansky and Scriber 1985). The initial larval percent dw was estimated ($n=20$) as described previously (Experiment 1). Insect feces were removed daily after the second day of the experiment and larvae were refed

as needed. Larval feces and uneaten diet were collected, dried and weighed. All data (randomized complete block experimental design) were analyzed with an ANOVA followed by the Tukey-Kramer test (Sokal and Rohlf 1981) using SAS/PC (SAS Institute, Inc., 1987), except for the mortality data which were analyzed with a G-test (Zar 1984).

Experiments 3a-3e. Influence of Greenhouse Induction of Soybean Defenses on Herbivore Performance

A series of experiments was conducted evaluating the activity of soybean foliar extract fractions from mite-free and mite-damaged foliage on larval mortality and RGR (calculated as in Experiment 2a). Several non-adapted and adapted soybean folivores were included, the velvetbean caterpillar, fall armyworm (FAW), Spodoptera frugiperda (J. E. Smith), the corn earworm (CEW) Heliothis zea (Boddie), the tobacco budworm (TBW) H. virescens F. and the cabbage looper (CL) Trichoplusia ni (Hübner), all obtained as eggs (from the USDA-ARS, Experiment 2b) and reared to the third instar before feeding on the treatment diets. With the exception of the velvetbean caterpillar and the corn earworm (primarily a pod-feeder), these species are not major soybean foliage pests (Kogan and Turnipseed 1987) and thus may not be as well adapted to the allelochemicals contained in soybean foliage. All studies involved rearing larvae for 3 d on the standard artificial diet (Experiment 2b) that including various soybean foliar extracts. Mortality data

were analyzed either by a probit analysis (Finney 1971) or a G-test (Zar 1984), whereas the RGR data (randomized complete block experimental design) were analyzed by ANOVA and means were separated with the Tukey-Kramer test using SAS/PC (SAS Institute, Inc., 1987).

Agronomic methods. The influence of piercing-sucking herbivore damage on the induction of soybean defenses was studied by evaluating herbivore mortality while feeding on diet containing extract fractions of the treatment foliage. Three soybean lines were evaluated, the two previously mentioned lines (Bragg and PI229358, Experiment 1), plus the advanced breeding line D75-10169 (whose pedigree comprises Govan x (Bragg x PI229358) (Hartwig and Edwards 1985). Plants were greenhouse grown ($27 \pm 5^{\circ}\text{C}$, $50 \pm 10\%$ RH), three to a 25.4 cm (10 in) plastic pot, in a sterilized soil mixture (soil, sand, vermiculite 2:1:1) until the R3-R4 phenophase (Fehr et al. 1971). Pots were fertilized (0-10-20) monthly until foliage harvest.

Induction of defenses. Soybean plants were infested with twospotted spider mites (Tetranychus urticae Koch) by moving plants into infested areas of the greenhouse. Only plants reaching high mite densities (where the webs covered nearly all the foliage) were used. Plants that did not support abundant mite populations were discarded. However, none of the Bragg nor PI229358 plants were successfully infested. Plants, designated as controls, were kept mite-

free by isolating them from the infested plants. Thus, the plant treatments consisted of mite-free susceptible Bragg, mite-free resistant PI229358, and mite-free and mite-damaged resistant D75-10169.

Extraction of foliage fractions. Leaves were hand-harvested, stored and extracted as described previously (Experiment 2b, Fig. 3-2). Because results in Experiment 2b indicated the benzene fraction was active, this fraction from the mite-free D75-10169 treatment was further purified by sequentially eluting with 5:95% acetone:benzene followed by 5:95% methanol:benzene on a silica gel (100-200 mesh, Fisher Scientific) column (15 x 225 mm). The eluate was separated into two fractions, the acetone and the methanol extractables. Each fraction included several bands that were monitored with thin layer chromatography (TLC, Kieselgel 60 HF, EM Science) using solvent mixtures ranging in polarity from 1:99% acetone:benzene to 5:95% methanol:chloroform. All fractions were rotoevaporated to dryness and refrigerated (10°C).

The active fractions (reported in Results) were further purified by ionization with 0.5N NaOH (pH=11-12) followed by extraction with chloroform. The ionizable fraction was neutralized (pH=7) with 1N HCl. The extracts were divided into the ionizable and non-ionizable fractions and developed with TLC (5:95% methanol: chloroform). The developed bands from the extracts were compared with the isoflavone

standards genistein, daidzein (both provided by S. K. Chattopadhyay, Department of Medicinal Chemistry, College of Pharmacy, University of Florida), coumestrol (Eastman Kodak Co.) rotenone and biochanin A and the flavones rutin and quercetin (Sigma Co.). Following development, flavonoid spots were viewed under short UV light (254 nm), sprayed with ferric chloride (5% dw dissolved in methanol) and heated on a hot plate set at low ($\pm 75^{\circ}\text{C}$) (Krebs et al. 1969) for phenolic visualization (Egger 1969). The R_f values were calculated by dividing the distance between the origin and the center of each flavonoid spot by the distance between the origin and the solvent front. Final purification was achieved by scraping individual bands developed with preparative TLC (25 x 25 cm, Kieselgel 60 HF, EM Science) run 3-5 times in 5:95% acetone:benzene.

Experiment 3a. Influence of Greenhouse Induction of Soybean Defense on Velvetbean Caterpillar Mortality

Treatments included the benzene, acetone and methanol fractions mixed with the standard artificial diet (Experiment 2b). The benzene and acetone fractions were mixed at concentrations ranging from 0.05-2% dw. The methanol fraction was incorporated at only the 2% dw concentration. The standard solvent + cellulose control diet was also included (Experiment 2b). Velvetbean caterpillar larval mortality was recorded.

Experiment 3b. Effect of Induced Resistance on Non-adapted and Adapted Soybean Herbivores

This study was designed to determine the influence of extracts from mite-free and mite-damaged treatments on non-adapted and adapted soybean herbivores. The benzene fractions from the mite-free and mite-damaged D75-10169 soybean line, were incorporated into the standard artificial diet (0.5% dw) and were fed to third instar larvae (20 replicates) of three noctuid species, the corn earworm, the fall armyworm and the tobacco budworm. Larval RGR data were calculated.

Experiment 3c. Sensitivity of Three Noctuid Species to the Non-benzene Soybean Foliar Extract Fractions

The previous two experiments (3a and 3b) only evaluated activity in the benzene extract fractions (the active fraction as demonstrated by the results of Experiment 2b) against non-adapted and adapted herbivore species. However, it is possible that an induced allelochemical is extracted in another solvent. Thus, the larvae of three noctuid species, velvetbean caterpillar, fall armyworm and cabbage looper were fed (20 replicates) artificial diets containing the non-benzene fractions (at 0.5 and 1% dw) from Bragg and mite-free and mite-damaged D75-10169 foliage. Larval RGR data were calculated.

Experiment 3d. Effect of the Petroleum Ether Extract Fraction on RGR of Non-adapted and Adapted Soybean Herbivores

This experiment was designed to confirm the reduction in RGR caused by the petroleum ether extract. Results from the previous study indicated that petroleum ether extractables of the mite-damaged D75-10169 treatment reduced herbivore RGR values significantly more than the comparable mite-free treatment. The same three herbivore species tested in Experiment 3c (velvetbean caterpillar, fall armyworm, cabbage looper) were fed artificial diets containing the petroleum ether extract fraction (at 0.5 and 1% dw) from the foliage of Bragg, mite-free and mite-damaged D75-10169, and an untreated control (20 replicates).

Experiment 3e. Purification of the Active Fractions

The Bragg benzene extract fraction (from Experiment 3a) was washed with sequentially increased concentrations of acetone (1:99% to 15:85% acetone:benzene) eluting seven fractions by following major bands on TLC (as described in Experiment 3a). These solvent-free fractions were incorporated into the standard artificial diet (at 0.5 and 1% dw) and fed to larvae (10 replicates) of 5 noctuid species; the velvetbean caterpillar, fall armyworm, cabbage looper, tobacco budworm and the corn earworm. Insufficient material was available to test fraction 21-23 at both levels, and thus it was tested at only the 0.5% (dw) concentration.

Results

Experiment 1. Induction of Susceptible and Resistant Soybean

Preliminary results of the effects of induced resistance suggest changes in larval performance when fed foliage from velvetbean caterpillar damaged plants. However, nearly half of the field collected larvae died from an outbreak of the entomopathogen Nomuraea rileyi (Farlow) Samson, and thus, no statistical analyses were conducted on these data. However, the results suggest that larval relative growth rates [$RGR = \text{biomass dw gain (mg)} / \text{average caterpillar dw (mg)} / \text{developmental time (d)}$] may have increased for both the velvetbean caterpillar and the soybean looper when fed the undamaged resistant (PI) foliage compared with the undamaged susceptible (Bragg) variety (Table 3-1). Additionally, velvetbean caterpillar and soybean looper larvae had reduced RGR values when fed foliage from damaged plants of resistant foliage compared with foliage from undamaged resistant plants. However, velvetbean caterpillar, soybean looper and green cloverworm larval RGR values increased when fed foliage from damaged plants of the susceptible Bragg cultivar. Furthermore, the RGR of green cloverworm larvae decreased dramatically on the resistant compared to the susceptible line. Further studies are required to determine the significance (statistical and/or biological) of these differences.

Table 3-1. Average (\pm se) relative growth rates (RGR) of velvetbean caterpillar, soybean looper and greenclover worm larvae fed field grown undamaged or damaged soybean foliage. Damaged treatments consisted of plants that had prior (48 h) velvetbean caterpillar larval damage. See Results (Experiment 1) for RGR formula.

Line ¹	Spp ²	Treat ³	n	RGR	se
BG	VBC	CHK	3	0.45	(± 0.16)
BG	VBC	DAM	3	0.75	(± 0.47)
PI	VBC	CHK	2	0.75	(± 0.19)
PI	VBC	DAM	3	0.56	(± 0.12)
BG	SBL	CHK	7	0.69	(± 0.13)
BG	SBL	DAM	8	0.93	(± 0.21)
PI	SBL	CHK	5	0.79	(± 0.24)
PI	SBL	DAM	6	0.68	(± 0.10)
BG	GCW	CHK	4	0.58	(± 0.40)
BG	GCW	DAM	3	0.83	(± 0.59)
PI	GCW	CHK	3	0.06	(± 0.04)
PI	GCW	DAM	4	0.06	(± 0.50)

¹ Lines: BG=Bragg (susceptible); PI=PI229358 (resistant).

² Species: VBC=velvetbean caterpillar; SBL=soybean looper; GCW=green cloverworm.

³ Damage treatments: CHK=undamaged plant; DAM=VBC damaged plant.

Experiment 2a. Preliminary Extract Methods

The average larval biomass gain and feces production were significantly influenced only by the extract fraction (e.g., control, water, etc.) but not by the concentration of each fraction ($P=0.40$). Thus, concentration effects were considered as replicates of each extract fraction. Biomass gain and feces production for larvae fed leaves treated with the petroleum ether fraction were significantly reduced (39

and 36%, respectively) compared with the control leaves (Table 3-2). Biomass gain of larvae feeding on the water fraction was also significantly greater than on the petroleum ether fraction. Furthermore, larvae feeding on the petroleum ether fraction produced less feces than the larvae feeding on the other treatments, possibly due to reduced consumption.

Table 3-2. Average (\pm se) biomass gain (dry weight=dw) and feces production by third instar soybean looper larvae fed field grown soybean foliage treated with soybean (Kirby) foliar extract fractions.

Fraction ¹	n	Biomass gain (dw, mg)	Feces (dw, mg)
Control	15	55.8 (\pm 4.7) a ²	194.8 (\pm 26.1) a
Water	45	49.8 (\pm 5.4) a	213.7 (\pm 15.3) a
Water (Hydrolyzed)	45	45.6 (\pm 4.0) ab	173.0 (\pm 14.5) a
Ethyl acetate	45	44.2 (\pm 5.4) ab	201.8 (\pm 25.2) a
Petroleum ether	45	33.8 (\pm 4.7) b	124.6 (\pm 16.8) b

¹ See text for descriptions.

² Means followed by the same letter are not significantly different according to a least square means test ($P=0.05$).

Experiment 2b. Refined Soybean Foliar Extraction

Survival of velvetbean caterpillar larvae feeding on the soybean foliar extract fractions was lowest on the benzene 1% (dw) diet (13%) compared with the other

treatments (Table 3-3). The benzene 0.5% and methanol 0.1% treatments were deleted from the G-test analysis because of zero mortality. Additionally, the RGR, efficiency of digestion and absorption of dw diet [$AD=100 \times (\text{ingestion} - \text{feces}) / \text{ingestion (all dw, mg)}$], efficiency of conversion of digested food into insect tissue [$ECD=100 \times \text{biomass gain} / (\text{ingestion} - \text{feces}) \text{ (all dw, mg)}$] and efficiency of converting ingested food into insect tissues [$ECI=100 \times \text{biomass gain} / \text{ingestion (all dw, mg)}$] values were reduced for the two survivors of the benzene extract (1%). The petroleum ether fractions, isolated from the preliminary study (PE1), are included here to compare with the toxicity of the more refined extract fractions.

Experiment 3a. Influence of Greenhouse Induction of Soybean Defense on Velvetbean Caterpillar Mortality

Percent mortality data suggest that mortality increased with greater concentrations of each benzene extract, regardless of the undamaged or damaged condition of the plants (Table 3-4). Because zero observations occurred in many cells (18 of 29 cells where mortality was either 0 or 100%), the fiducial limits of the probit analysis (which uses a χ^2 analysis) could not be confidently ($P=0.05$) estimated. However, concentration data for each plant extract could be combined to produce an overall percent mortality value that was analyzed with a G-test (Zar 1984).

Table 3-3. Mean nutritional parameters (\pm se) of third instar velvetbean caterpillar larvae reared for 5 d on artificial diets containing soybean (var. Kirby) foliar extracts. RCR=relative consumption rate; AD=assimilation efficiency; ECI=efficiency of conversion of ingested food; ECD=efficiency of conversion of digested food; RGR=relative growth rate; See Experiment 1 Results for RGR formula and Experiment 2b Results for remaining nutritional parameter equations.

Nutritional Parameters																
Frac- ¹ tion	Surv ² Conc (%)	RCR	se	AD	se	ECI	se	ECD	se	RGR	se					
WATER	1	67	1.64	(±0.06)	51.85	(±1.71)	a ³	31.43	(±1.53)	a	75.37	(±2.83)	a	0.51	(±0.01)	a
PE1	1	60	1.96	(±0.49)	49.09	(±5.20)	a	30.15	(±3.76)	a	68.38	(±9.13)	ab	0.47	(±0.04)	a
PE1	.5	53	1.51	(±0.05)	46.81	(±1.72)	ab	30.46	(±0.76)	a	65.66	(±2.89)	ab	0.46	(±0.02)	a
CONT	-	73	1.79	(±0.08)	46.55	(±1.67)	ab	29.58	(±1.00)	a	64.69	(±3.52)	ab	0.52	(±0.02)	a
PE	1	73	1.59	(±0.09)	46.72	(±3.48)	ab	28.14	(±2.75)	a	63.89	(±6.80)	abc	0.44	(±0.04)	a
PE	.5	87	1.78	(±0.05)	44.09	(±1.09)	ab	27.46	(±1.07)	a	62.81	(±2.81)	abc	0.49	(±0.02)	a
BENZ	.5	100	1.64	(±0.07)	43.54	(±1.51)	ab	26.51	(±0.97)	a	61.63	(±2.67)	abc	0.43	(±0.02)	a
METH	.1	100	1.84	(±0.08)	46.17	(±1.41)	ab	27.70	(±0.94)	a	60.09	(±1.15)	abc	0.50	(±0.01)	a
METH	.5	80	1.80	(±0.11)	47.12	(±1.47)	ab	27.54	(±1.24)	a	58.71	(±2.53)	abc	0.48	(±0.02)	a
WATER	.5	93	1.82	(±0.06)	50.24	(±2.11)	ab	27.54	(±0.69)	a	55.96	(±2.42)	bc	0.50	(±0.02)	a
BENZ	1	13	1.28	(±0.19)	29.32	(±9.04)	b	10.52	(±7.69)	b	30.71	(±16.76)	c	0.15	(±0.11)	b

¹ Fractions: CONT=control; PE1=petroleum ether from Experiment 2a; PE=petroleum ether; BENZ=benzene; METH=methanol.

² Percent survival of the treatments were significantly different according to a G-test ($\chi^2=71.7$, df=8, $P < 0.005$).

³ Means followed by the same letter within a column, or where no letter appears, are not different according to a Tukey-Kramer test ($P=0.05$).

Table 3-4. Velvetbean caterpillar third instar larval mortality fed soybean foliar benzene fractions incorporated into artificial diet. Foliar extracts: BG=Bragg (susceptible); PI=PI229358 (resistant); DIND=D75-10169 mite-damaged; DCON=D75-10169 mite-free; ACE=acetone fraction of the BG extract.

Concen- tration (% dw)	BG		PI		DIND		DCON		ACE	
	n	% mort	n	% mort	n	% mort	n	% mort	n	% mort
0.05	20	5	20	0	20	5	20	0	nt	nt ¹
0.10	10	0	30	3	30	3	30	0	10	10
0.25	10	60	10	30	10	0	10	0	10	80
0.50	23	100	23	100	24	100	25	96	10	90
1.00	35	43	20	100	14	100	14	100	20	100
2.00	9	100	13	100	12	100	12	100	20	100
Overall ² % mortality	50		52		47		45		83	

¹ nt=not tested.

² Overall mortality was significant according to a G-test ($\chi^2=17.7$, $df=4$, $P<0.005$).

Although significant differences in mortality occurred among the plant extracts, distinction among the treatments is not possible with this analysis; however, the data suggest that differences in mortality among the mite-damaged (DIND), mite-free (DCON & PI) resistant treatments and the susceptible variety (Bragg) were minimal (45-52%), whereas the majority of mortality (83%) occurred when larvae fed on the refined acetone treatment. These data indicate that the activity contained in the benzene fraction is acetone

extractable. Mortality among larvae fed the methanol fraction (2% dw) and the control diet (data not shown) was 0 and 10%, respectively.

Experiment 3b. Effect of Induced Resistance on Non-adapted and Adapted Soybean Herbivores

The RGR values for larvae of the corn earworm, fall armyworm and tobacco budworm fed artificial diets containing benzene extract fractions of the resistant mite-free (DCON) or the resistant mite-infested (DIND) foliage were reduced compared with the unadulterated diet (CONT, Table 3-5). However, no differences were found between the mite-free and mite-infested treatments for any of the three species. Additionally, larval mortality was relatively low in all treatments, although three tobacco budworm larvae died in the DIND treatment, as did one CONT treatment larva. Thus, these results indicate all three species are sensitive to an extractable substance in the resistant line (D75-10169), however, mite-damaged foliage did not influence RGR values.

Experiment 3c. Sensitivity of Three Noctuid Species to the Non-benzene Soybean Foliar Extract Fractions

The petroleum ether fraction (at 1% dw) from the mite-damaged D75-10169 plants significantly reduced (by 52%) fall armyworm larval RGR values compared with the control diet (Table 3-6). No other non-benzene fraction significantly reduced fall armyworm RGR values. Velvetbean caterpillars

Table 3-5. Average (\pm se) relative growth rates (RGR see Experiment 1 Results for equation) of 3 noctuid larval species reared on artificial diets containing foliar soybean benzene extractables (0.5% dw) from mite-free and mite-damaged D75-10169 plants (DCON and DIND, respectively) and an unadulterated control diet (CONT).

SPP ¹	Treat ²	n	RGR	se	
CEW	CONT	20	0.57 (\pm 0.04)	a	³
CEW	DCON	20	0.25 (\pm 0.03)	b	
CEW	DIND	20	0.30 (\pm 0.03)	b	
FAW	CONT	20	0.60 (\pm 0.03)	a	
FAW	DCON	20	0.12 (\pm 0.01)	b	
FAW	DIND	20	0.10 (\pm 0.01)	b	
TBW	CONT	19	0.65 (\pm 0.03)	a	
TBW	DCON	20	0.47 (\pm 0.04)	b	
TBW	DIND	17	0.51 (\pm 0.04)	b	

¹ SPP: CEW=corn earworm; FAW=fall armyworm; TBW=tobacco budworm.

² Treatments: CONT=unadulterated diet; DCON=D75-10169 mite-free; DIND=D75-10169 mite-damaged.

³ Means within each species followed by the same letter are not significantly different according to a Tukey-Kramer test ($P = 0.05$).

feeding on the D75-10169 mite-damaged treatment (1% dw) had RGR values significantly reduced relative to only the Bragg water and Bragg petroleum ether treatments (1% dw). The cabbage looper RGR values were not significantly affected by additions of the non-benzene extracts to diet.

Experiment 3d. Effect of the Petroleum Ether Extract Fraction on RGR of Non-adapted and Adapted Soybean Herbivores

Larvae of the fall armyworm and the velvetbean caterpillar had reduced RGR values when fed diets containing the highest concentrations (1% dw) of the petroleum ether fraction from the resistant (D75-10169) mite-damaged treatment compared with the control diet (Table 3-7). Furthermore, the RGR values of the fall armyworm were significantly reduced on the mite-damaged resistant treatment relative to the mite-free resistant treatment (both 1% dw). These data confirm earlier findings (Experiment 3c) that the petroleum ether extractables from mite-damaged resistant foliage reduced fall armyworm RGR values relative to the control. However, contrary to previous results (Experiment 3c), RGR values for the velvetbean caterpillar were reduced on the mite-damaged resistant treatment compared with the control. Additionally, as reported in Experiment 3c, the cabbage looper was not significantly affected by extract additions to their diet.

Table 3-6. Average (\pm se, n) relative growth rates (RGR see Experiment 1 methods for equation) of the larvae of three noctuid species reared for 3 days on soybean foliar fractions incorporated into artificial diet (at 0.5 and 1% dw).

TRT ²	FRAC ³	FAW ¹		VBC		CL	
		CONC (% dw)	RGR (\pm se, n)	RGR (\pm se, n)	RGR (\pm se, n)	RGR (\pm se, n)	RGR (\pm se, n)
BG	WATER	1.0	.57 (\pm 0.02, 20) a ⁴	.81 (\pm 0.03, 20) a	.59 (\pm 0.03, 20)		
BG	PE	1.0	.63 (\pm 0.02, 20) a	.80 (\pm 0.03, 20) a	.65 (\pm 0.02, 20)		
BG	PE	0.5	.61 (\pm 0.02, 20) a	.78 (\pm 0.03, 20) ab	.65 (\pm 0.02, 18)		
BG	MeOH	1.0	.58 (\pm 0.01, 20) a	.78 (\pm 0.03, 20) ab	.62 (\pm 0.02, 18)		
DIND	WATER	1.0	.61 (\pm 0.02, 20) a	.77 (\pm 0.03, 20) ab	.62 (\pm 0.02, 19)		
DIND	PE	0.5	.56 (\pm 0.02, 20) a	.73 (\pm 0.03, 20) ab	.59 (\pm 0.02, 20)		
CONT	-	-	.60 (\pm 0.03, 20) a	.73 (\pm 0.02, 20) ab	.65 (\pm 0.02, 19)		
DIND	MeOH	1.0	.60 (\pm 0.02, 20) a	.73 (\pm 0.02, 20) ab	.65 (\pm 0.04, 18)		
BG	MeOH	0.5	.54 (\pm 0.01, 20) a	.72 (\pm 0.02, 20) ab	.64 (\pm 0.02, 20)		
DIND	MeOH	0.5	.60 (\pm 0.02, 20) a	.71 (\pm 0.02, 20) ab	.62 (\pm 0.03, 20)		
DIND	WATER (HYD)	0.5	.61 (\pm 0.02, 20) a	.70 (\pm 0.02, 19) ab	.63 (\pm 0.02, 17)		
DIND	WATER (HYD)	1.0	.60 (\pm 0.01, 20) a	.70 (\pm 0.01, 20) ab	.59 (\pm 0.02, 20)		
BG	WATER (HYD)	0.5	.62 (\pm 0.02, 19) a	.69 (\pm 0.03, 20) ab	.64 (\pm 0.02, 20)		
BG	WATER (HYD)	1.0	.62 (\pm 0.01, 20) a	.69 (\pm 0.03, 20) ab	.66 (\pm 0.02, 20)		
DIND	WATER	0.5	.61 (\pm 0.02, 20) a	.69 (\pm 0.03, 20) ab	.60 (\pm 0.03, 20)		
BG	WATER	0.5	.54 (\pm 0.01, 20) a	.67 (\pm 0.03, 20) ab	.63 (\pm 0.02, 19)		
DIND	PE	1.0	.29 (\pm 0.02, 20) b	.65 (\pm 0.04, 20) b	.51 (\pm 0.03, 20)		

¹ Species: FAW=fall armyworm; VBC=velvetbean caterpillar; CL=cabbage looper.

² Treatment: BG=Bragg; Cont=unadulterated diet; DIND=D75-10169 mite-damaged.

³ Fractions: PE=petroleum ether; MeOH=methanol; WATER (HYD)=hydrolyzed water extract fraction.

⁴ Means within each species followed by the same letter, or where no letter appears, are not significantly different according to a Tukey-Kramer test ($P=0.05$).

Table 3-7. Larval relative growth rates of 3 noctuid species reared on petroleum ether fractions from soybean foliage.

TRT ²	VBC ¹			FAW		CL	
	CONC (% dw)	RGR	(±se,n)	RGR	(±se,n)	RGR	(±se,n)
DCON	0.5	0.86	(±0.02,20) a ³	0.68	(±0.02,20) a	0.63	(±0.04,19)
DIND	0.5	0.82	(±0.02,20) ab	0.69	(±0.02,20) a	0.74	(±0.04,18)
CONT	-	0.82	(±0.02,20) ab	0.62	(±0.03,20) ab	0.75	(±0.03,20)
BG	0.5	0.78	(±0.03,20) abc	0.67	(±0.02,20) a	0.63	(±0.03,19)
BG	1.0	0.78	(±0.03,20) abc	0.64	(±0.03,20) a	0.68	(±0.05,17)
DCON	1.0	0.75	(±0.02,20) bc	0.54	(±0.01,20) b	0.57	(±0.04,20)
DIND	1.0	0.71	(±0.02,20) c	0.45	(±0.02,20) c	0.72	(±0.02,18)

¹ Species: VBC=velvetbean caterpillar; FAW=fall armyworm; CL=cabbage looper.

² Treatments: DCON=D75-10169 mite-free; DIND=D75-10169 mite-damaged; CONT=unadulterated diet; BG=Bragg.

³ Means followed by the same letter, or where no letter appears, within each species are not significantly different according to a Tukey-Kramer test ($P = 0.05$).

Experiment 3e. Purification of the Active Fraction

Bioassays of the purified benzene fraction indicated that only two of the fractions influenced the performance of two of the five noctuid species tested (Table 3-8). Mortality of fall armyworm larvae was highest (60%) on the 1-3 fraction (1% dw), whereas zero mortality occurred in the other treatments for this species. RGR values were also significantly reduced for fall armyworm larvae fed both the 1-3 (1% dw) and 21-23 fractions (0.5%). Similarly, the velvetbean caterpillar RGR was reduced on these two treatments; however, survival was not significantly affected. Cabbage looper, tobacco budworm and corn earworm survival and RGR values were not affected by these fractions.

Fraction yields and identification. Extract fraction yields were determined as a percent of the total foliage dw. For the different fractions these averaged (\pm se, $n=8$): water 3.47% (± 0.77); hydrolyzed aqueous 0.71% (± 0.05), petroleum ether 1.6% (± 0.47); benzene 2.23% (± 0.77) and methanol 0.68% (± 0.13) of the foliage dw.

The spots developed with TLC visualized under UV (254 nm) light and with ferric chloride from the active D75-10169 (petroleum ether induced) and acetone fractions are listed in Fig. 3-3. Additional spots were developed in more and less polar solvents but are not listed here because they

Table 3-8. Average larval relative growth rates of 5 noctuid species¹ fed refined benzene extracts from Bragg soybean foliage incorporated into artificial diet. The benzene extract was washed with increasing proportions of acetone:benzene².

Frac ²	Conc (%dw)	VBC			FAW			CL			TBW			CEW		
		RGR	se	% Mort	RGR	se	% Mort	RGR	se	% Mort	RGR	se	% Mort	RGR	se	% Mort
CONT	-	0.74a ³	0.02	0	0.73a	0.02	0	0.50	0.03	0	0.55	0.05	0	0.63	0.05	0
4-7	0.5	0.69a	0.02	0	0.73a	0.02	0	0.50	0.03	0	0.51	0.04	0	0.61	0.03	0
1-3	0.5	0.64a	0.02	0	0.70a	0.02	0	0.50	0.03	0	0.49	0.04	0	0.59	0.03	0
24-35	0.5	0.72a	0.02	0	0.69a	0.02	0	0.47	0.02	0	0.51	0.03	0	0.48	0.05	0
8-20	1.0	0.72a	0.02	0	0.69a	0.02	0	0.52	0.03	0	0.48	0.02	0	0.60	0.04	0
4-7	1.0	0.68a	0.01	20	0.68a	0.01	0	0.51	0.02	0	0.51	0.02	0	0.63	0.04	0
24-35	1.0	0.68a	0.03	0	0.68a	0.01	0	0.50	0.02	0	0.47	0.04	0	0.57	0.04	0
8-20	0.5	0.71a	0.02	0	0.68a	0.02	0	0.49	0.02	0	0.40	0.04	0	0.55	0.04	0
36-52	0.5	0.64a	0.02	20	0.67a	0.02	0	0.51	0.04	0	0.50	0.03	0	0.52	0.04	0
53-69	1.0	0.71a	0.02	0	0.67a	0.02	0	0.48	0.03	0	0.44	0.04	0	0.55	0.06	10
53-69	0.5	0.71a	0.06	0	0.66a	0.03	0	0.53	0.02	0	0.52	0.04	0	0.56	0.05	0
36-52	1.0	0.64a	0.05	0	0.62ab	0.06	0	0.46	0.02	0	0.40	0.05	0	0.56	0.05	0
21-23	0.5	0.27 b	0.04	0	0.50 bc	0.02	0	0.48	0.01	0	0.45	0.03	0	0.52	0.05	0
1-3	1.0	0.29 b	0.04	10	0.44 c	0.04	60	0.47	0.02	0	0.49	0.02	0	0.46	0.03	0

¹ Species: VBC=velvetbean caterpillar; FAW=fall armyworm; CL=cabbage looper; TBW=tobacco budworm; and CEW=corn earworm.

² Fractions were the result of eluting with increasing proportion of acetone relative to benzene and are numbered from 1-69. Fractions were combined based on bands monitored with TLC.

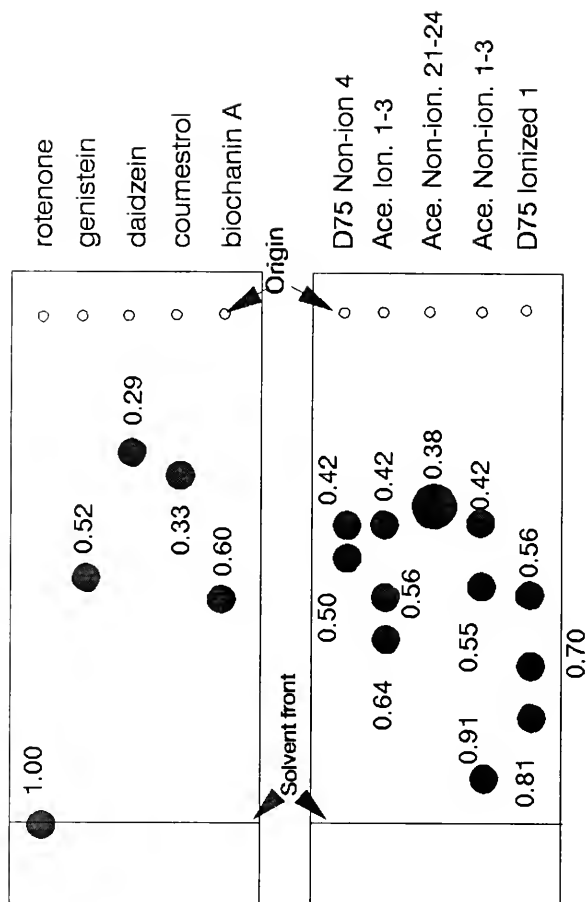
³ Means followed by the same letter, or where no letter appears, within each species are not significantly different according to a Tukey-Kramer test ($P=0.05$).

did not compare with the standards. Spots that developed in more polar solvents (20:80% methanol:chloroform) were compared on individual TLC plates with the flavones quercetin and rutin but none had similar R_f values (0.61 for quercetin, 0.80 for rutin). No spots were similar to rotenone.

The 0.38 spot (the largest spot developed) of the acetone non-ionized 21-23 fraction may have been similar to coumestrol (0.33); however, when chromatographed independently, the 0.38 spot of acetone non-ionized 21-23 had a greater R_f than coumestrol. The three spots that have R_f values of 0.42 may have been the same compounds, as were the spots at 0.55-0.56. These spots and the 0.50 spot of the D75 non-ionized 4 fraction, had R_f values similar to genistein (0.52). However, individual chromatographs indicated that genistein had a greater R_f value than the 0.50 spot and a smaller R_f value than the compounds at 0.55-0.56.

Although I know of no reports of the occurrence of biochanin A in soybean foliage, the spots at 0.55 (acetone non-ionized 1-3), 0.56, and 0.64 (acetone ionized 1-3) were indistinguishable from biochanin A (0.60) when chromatographed on individual TLC plates.

Fig. 3-3. Thin-layer chromatographs (Kieselgel 60 HF) visualized under UV light (254 nm) and by applying ferric chloride (10% in methanol). Spots were developed in 5:95% methanol:chloroform. The standards in the upper plate represent select soybean (coumestrol, daidzein and genistein) and other commercially available isoflavones (biochanin A and rotenone). The numbers adjacent to each spot represent the R_f values (distance between the origin and the center of each spot divided by the distance between the origin and the solvent front). All spots turned brown when sprayed with ferric chloride. Ace.=acetone, Ion.=ionized, Non-ion=non-ionized fractions.



UV absorbance peaks were recorded for D75 non-ionized 4 at 273 nm, acetone ionized and non-ionized 1-3 at 270 nm with a shoulder at 305, and D75 ion 3-4 at 265 and 275 nm; however little UV absorbance was found for acetone non-ionized 21-23. These may correspond with the UV absorbance peaks for many known soybean foliar isoflavones (Mabry et al. 1970); however these UV absorbance data do not match any published soybean foliar isoflavonoid data identically. Each fraction consisted of a mixture of compounds, undoubtedly several isoflavones and/or other UV absorbing compounds. This combination of several UV absorbing compounds could have produced the UV patterns recorded.

Discussion

Comparative relative growth rate values among studies.

In general, the RGR values obtained for the different herbivore species were greater than comparative published data calculated over the entire larval period. Data were collected here during the early instars (3-4), which have the greatest RGR values compared with later instars (Slansky and Scriber 1985). I chose this period because growth inhibitory substances are expected to have their greatest impact during this period. Additionally, instar three was the earliest instar during which reliable growth data could be collected with the methods used.

Larval RGR values for the velvetbean caterpillar may range from 0.31 to 0.40 on different host plant species (Slansky 1989) or from 0.38 to 0.50 on artificial diets (Slansky and Wheeler in press). Calculations of RGR values from published biomass gain data (Table 3-9) suggest that my values were similar to, or greater than, the results of other authors. The RGR for velvetbean caterpillar larvae reared on control diets for 3 days (e.g., Experiments 3c-3e) ranged from 0.62 to 0.82, whereas the RGR for 5 day studies ranged from 0.45 to 0.52 (Experiments 1, 2b).

The other herbivore species were tested only during a 3 day period and the RGR values were, as expected, greater than the results calculated from studies measuring growth during the entire larval period. The RGR values obtained for cabbage looper larvae (0.50 to 0.75, Experiments 3c-3e) were greater than the calculated RGR values from published data (0.23-0.50, Table 3-9). Similar results were found by calculating published fall armyworm growth data collected over the entire larval period (0.24-0.30, Table 3-9). The RGR for fall armyworm larvae may range from 0.30 to 0.43 on artificial diets for 5 day studies (Chapter 4) or from 0.22 to 0.26 on host plants during the entire larval phase (Crocomo and Parra 1985). The RGR values included here were consistently greater,

Table 3-9. Comparative larval developmental data for noctuid species reared on artificial diets and susceptible or resistant host plant foliage.

Diet	Pupal fresh wt. (mg)	Dry wt. ¹ gain (mg)	Larval devel. time (d)	Average ² dry wt. (mg)	RCR ³ (mg/mg/d)	Reference
<u>cabbage looper</u>						
ad ⁴	236.0	47.1	13.3	7.65	0.46	Canerday and Arant 1967
ad + pinitol	267.1	53.3	12.6	8.49	0.50	Gardner et al. 1984
ad + Clark 63	262.9	52.5	13.2	8.38	0.47	
ad	240.0	47.9	17.5	7.76	0.35	Khan et al. 1986
ad	267.0	53.3	17.3	8.49	0.36	
ad + PI227687	235.0	46.9	26.5	7.63	0.23	
ad + PI227687	287.0	57.3	17.3	9.02	0.37	
<u>corn earworm</u>						
PI229358	164.1	32.7	27.2	5.65	0.21	Hatchett et al.
PI229358	170.0	33.9	26.0	5.82	0.22	1976
Davis	224.5	44.8	21.4	7.34	0.29	
Davis	238.7	47.6	24.9	7.73	0.25	
Bragg	174.2	34.7	24.5	5.94	0.24	
Bragg	218.4	43.6	26.0	7.17	0.23	

1. Dry weight gain (mg) calculated by: (pupal fresh weight * 0.2) - initial dry weight (initial dry weight estimated=0.1).
2. Average larval dry weight (dw, mg) calculated by: final dw-initial dw)/(ln(final dw/initial dw)).
3. RGR=relative growth rate.
4. ad=artificial diet.

Table 3-9--continued.

Diet	Pupal fresh wt. (mg)	Dry wt. gain (mg)	Larval devel. time (d)	Average dry wt. (mg)	RGR (mg/mg/d)	Reference
D67.3297	366.0	73.2	17.7	11.10	0.37	Joshi 1981
Cutler	360.0	72.0	19.4	10.94	0.34	
Delmar	347.0	69.4	18.8	10.61	0.35	
Hill	319.0	63.8	18.7	9.88	0.35	
Essex	310.0	62.0	19.2	9.64	0.33	
York	300.0	60.0	19.3	9.38	0.33	
Wye	254.0	50.8	20.3	8.15	0.31	
Shore	244.0	48.8	21.1	7.88	0.29	
PI229358	207.0	41.4	21.8	6.87	0.28	Reese et al. 1982.
ad	-	31.5	11	5.48	0.52	
ad	-	69.7	11	10.65	0.60	
ad + pinitol	-	29.5	11	5.19	0.52	
ad + pinitol	-	29.1	11	5.13	0.52	Binder and Waliss 1984
ad + Frac A ⁵	398.6	79.7	17.3	11.93	0.39	
ad + Frac B	435.2	87.0	17.9	12.86	0.38	
ad + Frac C	418.9	83.8	17.4	12.45	0.39	
ad + Frac C	420.8	84.2	17.5	12.50	0.38	
ad + Frac D	425.7	85.1	18.0	12.62	0.37	
ad + Frac E	434.9	87.0	17.2	12.85	0.39	
ad + Frac F	345.2	69.0	21.2	10.56	0.31	
ad + Frac A-D	490.5	98.1	20.8	14.24	0.33	Gardner et al. 1984
ad + Frac E-F	349.4	69.9	22.0	10.67	0.30	
ad + Frac A-F	361.1	72.2	23.7	10.97	0.28	
ad	500.2	99.9	14.5	14.47	0.48	
ad + pinitol	511.7	102.2	15.7	14.75	0.44	

5. Frac.= Fractions: A=isoctane; B=ethyl acetate; C=acetone; D=methanol, ether soluble part; E=methanol, water-soluble part; F=water A+B+C+D.

Table 3-9--continued.

Diet	Pupal fresh wt. (mg)	Dry wt. gain (mg)	Larval devel. time (d)	Average dry wt. (mg)	RGR (mg/mg/d)	Reference
PI229358	73.8	14.7	14	2.94	0.36	Lambert and Kilen 1984
PI229358	40.8	8.1	14	1.84	0.31	
PI229358	70.0	13.9	14	2.82	0.35	
Davis	293.6	58.6	14	9.20	0.46	Gary et al 1985
Davis	336.4	67.2	14	10.32	0.46	
Davis	194.4	38.8	14	6.51	0.43	
PI229358	90.0	17.9	13	3.45	0.40	
Davis	286.8	57.3	13	9.02	0.49	
PI229358 gh ⁶ 82	38.7	7.6	14	1.76	0.31	Hart et al. 1988
PI229358 gh 83	5.9	1.1	14	0.45	0.17	
PI229358 f 83	54.9	10.9	14	2.32	0.33	
PI229358 intact	7.0	1.3	14	0.51	0.18	
PI229358 excised	15.0	2.9	14	0.86	0.24	
Forrest gh 82	90.0	17.9	14	3.45	0.37	Hatchett et al. 1976
Forrest gh 83	59.0	11.7	14	2.46	0.34	
Forrest f 83	49.0	9.7	14	2.12	0.33	
Forrest intact	72.0	14.3	14	2.88	0.35	
Forrest excised	37.0	7.3	14	1.70	0.31	
<u>tobacco budworm</u>						
PI229358	166.4	33.2	22.5	5.72	0.26	Hatchett et al. 1976
PI229358	128.5	25.6	25.3	4.62	0.22	
Davis	164.3	32.8	20.7	5.66	0.28	
Davis	189.3	37.8	18.8	6.36	0.32	
Bragg	164.5	32.8	20.0	5.66	0.29	
Bragg	176.8	35.3	20.4	6.01	0.29	

6. gh=green house or f=field grown.

Table 3-9--continued.

Diet	Pupal fresh wt. (mg)	Dry wt. gain (mg)	Larval devel. time (d)	Average dry wt. (mg)	RGR (mg/mg/d)	Reference
PI229358	24.4	4.8	18	1.24	0.21	Gary et al. 1985
Davis	96.4	19.2	18	3.65	0.29	
ad	304.1	60.7	13.2	9.47	0.49	
ad+caryophy.	279.6	55.8	14.7	8.83	0.43	Gunasena et al. 1988
<u>velvetbean caterpillar</u>						
PI229358	243.9	48.7	13	7.87	0.48	Gary et al. 1985
Davis	302.9	60.5	13	9.44	0.49	
Gasoy 17	70.9	14.2	2.4	-	0.53 ⁷	
PI 229358	57.4	11.5	3.0	-	0.36 ⁷	Beach and Todd 1988
GatIR 81-296	48.0	9.6	3.1	-	0.27 ⁷	
<u>fall armyworm</u>						
Gasoy 17	182.2	36.3	19.7	6.16	0.30	Beach and Todd 1987
GatIR 81-296	168.6	33.6	24.7	5.78	0.24	

7. Growth rate data provided by author (weight gain (dw, mg)/day).

ranging from 0.60 to 0.73 (Experiments 3b-3e). The corn earworm larval RGR values ranged from 0.57 to 0.63 (Experiments 3b, 3e), generally greater than the calculated values from published data (0.17 to 0.60, Table 3-9). Similarly, the RGR results obtained for tobacco budworm larvae were greater (0.55-0.65, Experiments 3b, 3e) than the calculated values from published data (0.21-0.49, Table 3-9). The RGR values published from rearing tobacco budworm larvae during the second instar on different cotton tissues (0.42-0.83; Mulrooney et al. 1985) include the range of data from my 3 day experiments.

Species Sensitivity to Constitutive Soybean Allelochemicals

Relative growth rate. My data indicated that all three species tested were sensitive to the benzene extractables in the resistant foliage. Reductions in RGR were detected for the fall armyworm (80%, Experiment 3b), the corn earworm (56%, Experiment 3b), and the tobacco budworm (25%, Experiment 3b) fed benzene extracts, from D75-10169 at 1% dw compared with the control. The velvetbean caterpillar larvae were equally sensitive (as indicated by mortality data, Experiment 3a) to the benzene extracts from the susceptible and resistant lines and this species had RGR values 71% lower than the control (Experiment 2b). The cabbage looper was tested only on the petroleum ether

fraction and the refined acetone extract, to which the species showed no sensitivity (Experiments 3c-3e).

However, the species surveyed in Table 3-9 were not consistent in their sensitivity to the resistant soybean foliage. The cabbage looper larvae, reared on diets containing leaf powder from a resistant soybean (PI227687), had RGR values 0 to 34% lower than the controls (artificial diet alone or including the leaf material from the susceptible Clark 63, Table 3-9). Additionally, the RGR values were reduced for larvae of the corn earworm (0 to 51%), the tobacco budworm (7-31%), the velvetbean caterpillar (2-49%), and the fall armyworm (20%) when reared on foliage from resistant lines (e.g., PI229358, GatIR 81-296) compared with foliage from susceptible lines (e.g., Bragg, Davis, D67.3297, Forrest, GaSoy 17) (Table 3-9).

The inconsistencies in RGR reduction presented in Table 3-9 may be due to a breakdown in resistance when the plants were grown under a 24 h compared with a 16 h photophase (Khan et al. 1986), or under shaded conditions (Hart et al. 1988) or when excised leaves were used instead of intact leaves (Hart et al. 1988). Furthermore, resistance may not be expressed when the larvae are reared for 24 h on a control diet prior to rearing on a resistant treatment (Reese et al. 1982).

The RGR incorporates biomass gain, per average larval biomass over a developmental period into a single variable.

Since it is a relative index, it is useful in comparing data from different studies on the same species where the larval weight may vary or among different species of different biomass. But in some instances, the RGR values may not reflect the degree of resistance found when comparing larval biomass gain (fw). In many cases the calculated RGR values listed in Table 3-9, may show little difference in magnitude among treatments, whereas the larvae reared from these treatments were much different in weight. Additionally, many studies measure biomass gain up to a particular day of development or to pupation (Lambert and Kilen 1984, Gary et al. 1985, Hart et al. 1988). Biomass differences may occur at one point during development but the larvae are able to overcome adverse treatment effects and pupate with the same weight as the controls (Hatchett et al. 1976). Where total developmental time was not included in the reviewed studies, the RGR values presented in Table 3-9 were calculated with development up to a particular day. My own data were calculated in this same manner, but I had the entire range of data to compare treatments statistically. I also analyzed the biomass gain (dw) data from my own studies, but did not include them here as the results duplicated the RGR results.

Mortality. Although unaccounted mortality was high in most of the studies reviewed in Table 3-9, it was generally higher for the resistant cultivars than the susceptible

controls. Mortality on the resistant foliage for the corn earworm was 7.5-93 (Beland and Hatchett 1976, Hatchett et al. 1976), for the tobacco budworm 47-63 (Hatchett et al. 1976), the velvetbean caterpillar 8-15 (Gary et al. 1985, Beach and Todd 1988) and for the fall armyworm 50 percentage points higher (Beach and Todd 1987) than when fed the susceptible cultivars. Mortality was significant in my studies only when the velvetbean caterpillar larvae fed on diets containing more than 0.25% dw of the benzene fraction (Experiments 2b, 3a) and when the fall armyworm larvae fed on diets containing the refined extract fractions (1-3 at 1% dw, Experiment 3e). The lack of sensitivity of the other species (i.e., cabbage looper, corn earworm, tobacco budworm) was unexplained by my data. Furthermore, these data do not agree with other reports of high mortality when rearing these species on resistant soybean foliage (e.g., Beland and Hatchett 1976, Hatchett et al. 1976). Bioassays using artificial diets that contain greater protein content than normally encountered in a foliage diet may have reduced sensitivity to allelochemicals (Rose et al. 1988). However, corn earworm larvae had 90% mortality when fed artificial diets containing soybean extract fractions (Binder and Waiss 1984). Additionally, partitioning the soybean foliar fractions and testing each separately may have reduced the overall toxicity of the material as combinations of the fractions may have been most toxic (Binder and Waiss 1984).

Velvetbean caterpillar larvae were sensitive to lower concentrations of extracted soybean foliar material incorporated into artificial diet than the levels found naturally in soybean leaves. Benzene extractables constituted 2.2% of the foliage dw, yet artificial diet formulations that included more than 0.25% dw were highly toxic to the velvetbean caterpillar. Allelochemicals incorporated into artificial diets (even though they may be adsorbed onto cellulose) may be more accessible to larval midgut digestion and absorption than compounds occurring naturally in foliage. Additionally, the larvae used in nearly all studies were from an inbred laboratory colony (80-100 generations); selection pressure on larvae for allelochemical tolerance fed artificial diet under laboratory conditions may have been much less than wild populations fed soybean and alternate host species.

Consumption rates and feeding efficiencies. The RCR and feeding efficiency data from the control treatment of Experiment 2b fall within the range presented in other studies using similar laboratory reared velvetbean caterpillar larvae on artificial diet (Slansky and Wheeler in press). The RCR values included here were not significantly affected by the extract fractions. Larvae of the soybean looper, however, decreased RCR 4-fold when fed resistant foliage (PI227687) compared with the susceptible control (Davis; Reynolds et al. 1984). Soybean looper

larvae feeding on herbivore damaged leaves also had reduced consumption (see Constitutive and induced resistance below). However, velvetbean caterpillar larvae (fifth instar - prepupa) feeding on resistant (PI229358 and GatIR 81-296) soybean foliage consumed the same amount (fw) or more than larvae feeding on the susceptible foliage (Beach and Todd 1988). Reasons for this difference may include herbivore species differences in response to these toxins (e.g., the velvetbean caterpillar larvae may not recognize the active component of soybean foliage). Although the velvetbean caterpillar larvae are probably soybean-adapted (or at least Papilionoideae-adapted, Chapter 2), they may fail to respond adaptively to the allelochemicals extracted in the benzene fraction by lowering consumption in order to avoid ingestion of a toxic dose (Slansky and Wheeler in prep.) or by inducing detoxication enzymes (Ahmad et al. 1986). Detoxication enzyme activity (mixed function oxidase) increased 1.5- and 1.8-fold when velvetbean caterpillar larvae were fed soybean foliage or diet containing commercial flavone over larvae fed unadulterated artificial diet (see Detoxication of soybean allelochemicals below) (Christian and Yu 1986). Additionally, the bioassay of individual components of the foliar extract may have separated the toxin(s) from the cue(s) recognized by the velvetbean caterpillar to respond adaptively.

The efficiency of digestion and absorption of nutrients (AD) was reduced (47% of the control diet) in larvae fed diet containing the benzene (1%) fraction. The velvetbean caterpillar larvae tested were undoubtedly stressed by the benzene treatment (the RGR value was 29% of the control) and thus the reduced AD may have resulted from less digestive enzymes available for nutrient digestion and absorption. Alternatively, the soybean allelochemicals may interfere with the activity of digestive enzymes.

The efficiency of conversion of ingested food (ECI) and digested food (ECD) decreased on the benzene (1% dw) diet to about one-third and one-half the level of the controls, respectively. Comparative data indicate no change occurred in ECI for fifth to prepupal velvetbean caterpillar fed resistant (PI229358, GatIR 81-296) cultivars; however, a significant reduction occurred during the ultimate stadium compared with larvae fed the susceptible line (Beach and Todd 1988). Similar reductions in ECI and ECD were reported for soybean looper larvae fed resistant (PI227687) compared with susceptible foliage (Beach and Todd 1988).

Detoxication of soybean allelochemicals. The mixed function oxidase system (MFO) plays an important role in insects in the metabolism of xenobiotics (e.g., insecticides, allelochemicals; Hodgson 1983) and endogenous compounds (e.g., hormones; Yu and Terriere 1978). Larvae of the velvetbean caterpillar, fall armyworm, and cabbage

looper increase their microsomal oxidase activity when fed alternate host plants (Christian and Yu 1986, Yu 1982, Fransworth et al. 1981). Preliminary data, included here in Appendices A-C because of the need for further replication, suggest that the larvae of these three noctuid species have different capabilities of oxidizing dietary soybean allelochemicals.

The oxidase activity of sixth instar velvetbean caterpillar larvae fed sublethal doses of the benzene fraction from the susceptible soybean line (Bragg) incorporated in artificial diet was about the same as larvae fed artificial diet alone (Appendix A). Larvae fed diets containing the petroleum ether fraction from the resistant line (D75-10169), at the 1% dw concentration, had ca. 75% of the oxidase activity of the control larvae. Fall armyworm larvae had higher oxidase activity when fed both the Bragg (32 percentage points higher) and D75-10169 mite-damaged (0.5% dw) (47 percentage points higher) treatments compared with the control. Fall armyworm larvae fed the highest concentration of the D75-10169 mite-damaged (1.0% dw) treatment had reduced oxidase activity compared with the control larvae.

Little change in oxidase activity was found again when either species (velvetbean caterpillar or fall armyworm) was fed diets containing commercial coumestrol or the benzene fraction (Bragg) at a range of concentrations (Appendix B).

An increase (30 percentage points) in oxidase activity occurred when velvetbean caterpillars were fed the highest concentration of the benzene treatment compared with the control larvae; however this difference is not consistent with the previous data (Appendix A) where no difference occurred for this species fed this fraction. Furthermore, the fall armyworm oxidase activity was reduced (25 percentage points) when fed the 0.25% dw coumestrol and the 0.25% dw benzene diets compared with the control; however no dose response was found in these treatments (i.e., greater activity at higher concentrations of allelochemical).

Induction of higher levels of oxidase activity occurred in the purified acetone fractions 1-3 and 21-23 for the fall armyworm and the cabbage looper (Appendix C). The acetone 1-3 fraction (at 1% dw) induced 2.7- and 5.2-fold increases in oxidase activity compared with the controls for fall armyworm and cabbage looper larvae, respectively. The lower concentration of the acetone 1-3 fraction and the acetone 21-23 fraction also induced higher levels in these two species. The oxidase activity of velvetbean caterpillar larvae was reduced on the acetone 1-3 fraction at the 0.5 and 1% concentrations (36 and 50% of the controls, respectively).

These results indicate that although the velvetbean caterpillar is sensitive to the mite-free susceptible and mite-damaged resistant soybean foliar extract fractions,

little induction in larval detoxication enzyme system occurred. Furthermore, the soybean foliar isoflavone, coumestrol, had little effect on enzyme activity in this species. However, cabbage looper larvae fed all soybean foliar fractions had little change in RGR values, perhaps due to the induction of detoxication enzymes, as demonstrated here on the purified acetone fractions. Further studies are required to determine whether MFO enzyme induction occurs with other fractions for this species.

The fall armyworm larvae also increased detoxication levels on these diets similar to the cabbage looper but to a lesser degree and perhaps, consequently had reduced RGR values (acetone 21-23) and greater mortality (acetone 1-3 at 1% dw). The velvetbean caterpillar larval detoxication system may have been inhibited by the acetone fraction 1-3 at both concentrations or, at least for the acetone 1-3 at the 1% dw treatment the larvae may have been too debilitated to induce greater enzyme activity.

Chemical mechanisms of soybean foliar resistance. The bioassay of various soybean foliage extractables has produced several conflicting reports; much of this information was reviewed by Smith (1985). Foliar sterols were one of the first discovered active components of resistant (PI229358) soybean foliage (Tester 1977), but the activity of these sterols against soybean herbivores could

not be confirmed (Grunwald and Kogan 1981). A larval growth inhibitor, pinitol, was found in the foliage of resistant (PI229358) soybean foliage (Dreyer et al. 1979), but similarly, its activity against herbivores could not be confirmed (see Reese et al. 1982, Gardner et al. 1984). Additional activity has been reported in sequentially extracted foliage (resistant) in the hexane, and ethyl acetate fractions (Khan et al. 1986). Other workers have discovered activity in the methanol fraction (after washes with petroleum ether and chloroform) (Smith and Fischer 1983). The most active compounds were reported from this methanol fraction; the flavonoids phaseol and afrormosin caused 71 and 98% mortality, respectively, in larvae of the soybean looper (Caballero et al. 1986). I was able to eliminate the possibility of the occurrence of several isoflavones (coumestrol, daidzein, genistein) from the active soybean foliar fractions. However, further purification and description of the individual components of these active fractions is needed for positive determination.

Induced resistance. Induced resistance has been elicited by lepidopteran larval (Haukioja and Niemelä 1977, Wallner and Walton 1979) and mite feeding damage (Karban and Carey 1984 and Harrison and Karban 1986, Karban 1988) and by cicada oviposition (Karban 1983). Spodoptera exigua larvae feeding on mite-damaged (T. turkestanii) cotton plants had reduced survival and extended life spans compared to larvae

feeding on unexposed plants (Karban et al. 1987, Karban 1988).

Induced resistance has been elicited by irradiation of soybean cotyledons with UV light (Hart et al. 1983), by damaging leaves with a rat-tail file (Reynolds and Smith 1985) or by damage caused by the Mexican bean beetle, Epilachna varivestis Mulsant (Chiang et al. 1987). The results of these studies suggested that induced resistance in soybean increased the foliar levels of total phenolic compounds (Chiang et al. 1987), decreased Mexican bean beetle feeding by 31-43% (Chiang et al. 1987), deterred Mexican bean beetle feeding (Hart et al. 1983) and decreased the soybean looper growth rate by 34-61% (Reynolds and Smith 1985). I also found reduced RGR values when fall armyworm (Experiments 3c and 3d), and in one case (Experiment 3d), velvetbean caterpillar larvae fed on a petroleum ether fraction, extracted from induced plants (52-27% and 13% reductions, respectively). However, the levels of resistance were minor compared with the constitutive levels detected in the benzene fraction (71% reduction in RGR and a 60 percentage point increase in mortality). Furthermore, the induced treatments did not influence RGR values for the other herbivore species tested. Although the comparisons of mite-free and mite-infested treatments directly examined the influence of mite damage on subsequent herbivory, in discarding plants that did not support mite populations, I

may have inadvertently excluded plants from the study that had high levels of induced resistance. These plants were never assayed chemically or biologically and therefore I have no way of knowing their level of resistance.

Potted greenhouse tobacco plants failed to exhibit the same induction of higher levels of foliar alkaloids as tobacco that was not pot-bound (Baldwin 1988). However, Chiang et al. (1987) detected induced resistance (higher plant enzyme activity) from potted greenhouse grown soybean plants. The failure to detect high levels of induced resistance in my foliar extracts and replicate reports of induction in foliage, may have several causes not addressed in my studies.

Conclusions

Most herbivore species tested were sensitive to the mite-free (constitutive) extracts from the resistant soybean foliage. Only the fall armyworm had significantly increased sensitivity (reduced RGR values) to the mite-damaged (induced) treatments compared with the mite-free treatment. This inducible treatment was petroleum ether extractable, contrasted by the benzene extractable constitutive treatment. However, the cabbage looper larvae were not significantly affected by either mite-free or mite-damaged treatments. The remaining species, the velvetbean caterpillar, fall armyworm, corn earworm, and tobacco

budworm had reduced RGR values when fed diets containing the mite-free (constitutive) resistant extracts. Additionally, velvetbean caterpillar larvae fed diets containing the benzene fraction from susceptible soybean foliage had reduced feeding efficiencies, but RCR values did not change significantly.

CHAPTER 4
COMPENSATORY RESPONSES OF THE FALL ARMYWORM (SPODOPTERA
FRUGIPERDA) WHEN FED WATER- AND CELLULOSE-DILUTED DIETS

Introduction

Insect herbivores have been shown to alter their feeding behavior (Simpson and Simpson in press), selection of dietary components (Waldbauer and Friedman 1988) and food utilization and growth (Slansky and Scriber 1985) in response to variations in food quality. Modifications in performance that may occur when fed diets with altered nutrient content include increases in feeding rate (Scriber 1977; 1979; Scriber and Feeny 1979; Timmins et al. 1988; Slansky and Wheeler 1989; in press), efficiency of converting food to insect tissue (House 1969; Slansky and Wheeler 1989; in press), or digestion and absorption of the ingested nutrients (Scriber 1979; Slansky and Wheeler 1989; in press). Despite these apparently compensatory responses, weight gain may be reduced because the insects are not able to fully compensate for the suboptimal conditions, or because of increased metabolic costs associated with increased food intake and processing (Timmins et al. 1988; Slansky and Wheeler 1989; in press).

The importance of water balance in the Lepidoptera has been demonstrated by rearing caterpillars on dry diets or under arid conditions (Fraenkel and Blewett 1944; Scriber 1977; Scriber and Feeny 1979). Below about 60-65% foliar water content, caterpillar growth declines due to increased metabolic costs associated with water limitation (Scriber 1977, Van't Hof and Martin 1989). Most commonly, low dietary moisture decreases caterpillar feeding efficiencies (Scriber 1977; Reese and Beck 1978). Too much dietary water may also have a negative impact on caterpillar performance (Reese and Beck 1978; Timmins et al. 1988). However, simultaneous variation in nutritional factors of natural hosts, such as foliar water and nitrogen content, make it difficult to determine the cause of a change in insect performance (Scriber 1979).

Spodoptera frugiperda (J.E. Smith) is potentially a very polyphagous herbivore, capable of feeding on a diverse array of plant species; however, the caterpillars feed primarily on graminaceous species (Luginbill 1928; Ashley et al. 1989). Nutritional studies on other Spodoptera spp. describe larval feeding responses to different host species (Soo Hoo and Fraenkel 1966; Scriber 1979), qualitatively altered host plants (Al-Zubaidi and Capinera 1984; Manuwoto and Scriber 1985a; 1985b) and adulterated artificial diets (Johnson and Bentley 1988; Peterson et al. 1988; Simpson et al. 1988). Although some studies have addressed the

performance of S. frugiperda caterpillars on various host plant varieties (Quisenberry and Wilson 1985; Lynch et al. 1986, Chang et al. 1987; Jamjanya and Quisenberry 1988) or species (Crocomo and Parra 1985), little is known about the factors affecting their consumption and utilization of food. In the present study, I investigated the compensatory feeding responses of S. frugiperda caterpillars to diets diluted with water and cellulose, and the impact of these responses on caterpillar performance. This information will allow us to gain a better understanding of the regulation of feeding and the factors affecting post-ingestive food utilization in foliage-feeding caterpillars.

Materials and Methods

Diets

A standard artificial diet (Greene et al. 1976, lacking formalin and tetracycline) was modified by incorporating different amounts of water and cellulose (alphacel, ICN Biochemicals Inc.) to obtain different nutrient levels (Table 4-1). The undiluted diet (# 1) was formulated to contain no added cellulose and 68% fresh weight (fw) water (i.e., 32% fw nutrients). By increasing the cellulose or water contents, two diets were formulated with 19% fw nutrients (diets # 2 and 4, respectively) and three were formulated with 10% fw nutrients by addition of cellulose

(# 3), water and cellulose (# 5) or water alone (# 6). The range of dietary water content represents the extremes of low and high moisture found in representative host plants of S. frugiperda (Chang et al. 1987); while the range of cellulose content [41 to 69% dry weight (dw)] exceeds that normally found in forage crops (Jamjanya and Quisenberry 1988), it along with other indigestible materials (especially lignin and hemicellulose) includes the levels found in other foliage types (Van Soest 1982). The levels of cellulose describe the amount added to the standard diet whose ingredients (e.g., wheat germ and pinto beans) may contain some indigestible material; thus, the 'nutrient' level describes the amount of diet ingredients added to the formulations and not the absolute amount of nutrients. All prepared diets were stored in sealed petri dishes at 10°C until use. Immediately prior to each feeding, 5-10 samples (each ca. 500 mg) of each diet were weighed fresh, dried at 60°C for 48 h and reweighed to estimate the % dw. This dw conversion value was used to estimate the dw of diet given to the caterpillars as part of calculating dw food consumption (see below).

Insects

Insect eggs were obtained from the Insect Attractants, Behavior, and Basic Biology Research Laboratory, USDA/ARS, Gainesville, Florida. The egg stage through the third

instar occurred en mass within 24 X 24 X 11 cm refrigerator boxes on the standard artificial diet (# 4). Fifteen third instars (10-25 mg fw) were transferred to each treatment diet and reared to pupation. Each experimental insect was reared individually in an inverted 30 ml plastic cup with a waxed lid. Each cup contained (in addition to the larva) a pre-weighed portion of diet and a 2 ml layer on the cup top (the base in an upright cup) consisting of 1.5% fw Gelcarin HWG (Marine Colloids, Inc., 2 Edison Place, Springfield, NJ, USA 07081), to maintain moisture. A subset (n=10) of the same caterpillar cohort was weighed fresh and dried (as above) to estimate initial dw. After day 2, caterpillars were refed and feces were removed daily. At the pupal stage, each individual was weighed fresh, frozen and dried along with any uneaten diet and the total feces produced to obtain their respective dry weights.

Analyses

Nutritional indices were calculated on a dw basis by a gravimetric technique (Waldbauer 1968; Slansky 1985; Slansky and Scriber 1985; formulae given in **Results**). Additionally, fw consumption was calculated by dividing each caterpillar's dw consumption by the % dw of its diet. Lipid content of pupae and diet was determined by dw loss following 3 h Soxhlet extraction with petroleum ether (Atkins 1969). Lipid-free weight gain was calculated by subtracting the

pupal lipid content from the final pupal dw. Energy content of the diets and caterpillars was calculated by summing the energy contained in the lipid and non-lipid components, based on standard conversion values (Hochachka and Somero 1984) for lipid ($39.58 \text{ J mg}^{-1} \text{ dw}$) and combined protein and carbohydrate (carbohydrate= $17.49 \text{ J mg}^{-1} \text{ dw}$, protein= $18.08 \text{ J mg}^{-1} \text{ dw}$, combined non-lipid value= $17.79 \text{ J mg}^{-1} \text{ dw}$). The absorption of energy was calculated by multiplying the energy content of the ingested food ($18.7 \text{ J mg}^{-1} \text{ dw}$) by the approximate digestibility for nutrients (ADNU) (see **Results** for equation of ADNU).

Percent survival data were analyzed with a G-test and the slopes from linear regressions were compared with a Student's t test (Zar 1984). All other analyses [ANOVA, Tukey-Kramer test ($P=0.05$), Student's t test, Pearson correlation (Sokal and Rohlf 1981), and simple linear, polynomial and stepwise multiple regressions (Draper and Smith 1981)] were conducted with SAS/PC (SAS Institute, Inc. 1987). Quadratic regression equations were used when the second-order parameter estimate was significant ($P < 0.05$).

Results

Diets

As planned, the diets fell into three distinct groups (diets 1-3 ca. 32% dw; diets 4-5, 19% dw; and diet 6, 10% dw; Table 4-2). Although there were significant differences

within the 32% dw group (probably due to slight variation in measurements of the dietary ingredients or to different periods during which the containers were open while dispensing the diet such that additional moisture may have been absorbed from the air), these differences were slight ($< 7\%$ of the % dw of the diets) and are considered of minimal biological relevance as suggested by previous studies (Slansky and Wheeler 1989; in press). The diet formulations comprised 3 distinct nutrient levels (diet 1 ca. 32% fw; diets 2 and 4, 19% fw; and diets 3, 5 and 6, 10% fw; Table 4-1).

Larval Mortality and Developmental Time

Only 2 caterpillars died (diets 3 and 4) prior to the prepupal stage; although mortality increased between the prepupal and pupal stages (Table 4-2), there were no significant differences among treatments ($G=6.35$, $df=5$, $0.25 < P < 0.5$). Developmental time to the prepupal stadium changed little with diet dilution. Caterpillars reared on the 10% nutrient content diet diluted only with water (# 6) required more time than those on all other diets (Table 4-2). No other diets produced significantly different developmental times.

Larval Composition

The estimated initial caterpillar dw averaged (\pm SE) 4.1 (\pm 0.08) mg and did not differ significantly among treatments ($P=0.16$). Initial caterpillar water content was 80.7% (\pm 0.01) fw and at the end of the experiment pupal water content varied little among the diets (Table 4-2). Pupal water content was greatest on the wettest diet (# 6) compared with that of pupae reared on diet 2.

Table 4-1. Planned cellulose, water, 'nutrients'¹ and lipid² contents of the diets for Spodoptera frugiperda caterpillar rearing.

Diets	Cellulose (% fw)	Water (% fw)	Nutrients (% fw)	Lipid (% fw)
(1)	0	68	32	1.51
(2)	12.9	68	19	0.90
(3)	21.9	68	10	0.47
(4)	0	81	19	0.90
(5)	9.0	81	10	0.47
(6)	0	90	10	0.47

¹ 'Nutrients' refers to the normal dietary ingredients, which include naturally occurring indigestible material.

² The dietary lipid content was measured for diet 4 ($0.90 \pm 0.17\%$ fw, $n=4$) and calculated for the remaining diets based upon the cellulose and water dilutions.

Table 4-2. Mean (\pm SE) values for 6 diets fed to Spodoptera frugiperda caterpillar instars 3 to pupation. Means represent 15 (diets #1, 2, 5, and 6), 14 (#4), and 10 (#3) caterpillars. Data for diet # 4 included 14 caterpillars due to mortality of one individual. Data for diet # 3 included 10 caterpillars due to mortality of one and loss of the feces of four other individuals. Means within a measure followed by the same letter are not significantly different according to a Tukey-Kramer procedure ($P=0.05$) * or a G test **.

Measure	Diets					
	1	2	3	4	5	6
Diet % dw *	31.4 b (\pm .2)	30.5 c (\pm .1)	32.8 a (\pm .1)	18.9 d (\pm .2)	19.1 d (\pm .1)	10.0 e (\pm .1)
Mortality ** to pupa	5	4	3	2	1	1 ns
Time to*	6.5 b	6.5 b	6.6 b	6.4 b	6.6 b	7.4 a
prepupa (d)	(\pm .2)	(\pm .1)	(\pm .2)	(\pm .1)	(\pm .2)	(\pm .3)

Table 4-2--continued. Diets

Measure	1	2	3	4	5	6
Pupal water	74.8 ab	73.6 b	75.7 ab	75.4 ab	75.6 ab	76.9 a
(% fw) *	(± 0.6)	(± 0.5)	(± 0.5)	(± 0.7)	(± 0.6)	(± 0.6)
Final pupal	53.4 ab	58.3 a	53.0 ab	47.4 b	47.6 b	34.6 c
dw (mg) *	(± 1.8)	(± 1.9)	(± 2.2)	(± 1.4)	(± 3.0)	(± 0.8)
ADDW (%) *	37.7 c	25.8 d	15.0 e	41.6 b	24.5 d	48.7 a
	(± 1.3)	(± 0.5)	(± 0.9)	(± 1.4)	(± 0.9)	(± 1.2)
ECIDW (%) *	19.4 a	15.6 b	7.8 c	19.7 a	11.3 c	17.4 ab
	(± 0.7)	(± 0.3)	(± 0.2)	(± 0.9)	(± 0.4)	(± 0.8)
ECINU (%) *	19.4 cd	27.1 a	23.5 b	19.7 cd	21.3 bc	17.4 d
	(± 0.7)	(± 0.5)	(± 0.5)	(± 0.9)	(± 0.7)	(± 0.8)

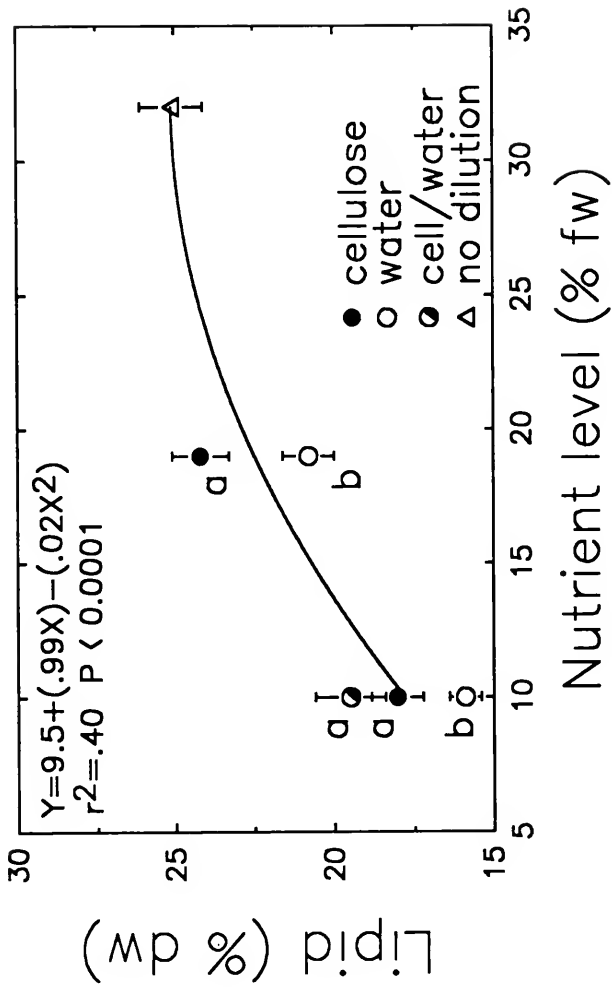
Pupal lipid content was positively related to diet nutrient level (Fig. 4-1). At the 19% nutrient level, the cellulose-diluted diet (# 2) produced pupae with significantly higher lipid content ($P < 0.01$, $t=2.71$, $df=28$) than pupae from the comparable diet diluted with water (# 4). On the 10% diets, the diet diluted exclusively with water (# 6) had lower pupal lipid content than the other two (# 3 and 5).

Pupal lipid content was significantly correlated with pupal lipid-free dw only on the highest cellulose (# 3) and water (# 6) dilutions ($r=0.54$, $P < 0.05$ and $r=0.52$, $P < 0.05$, respectively). These results suggest that the caterpillars which were able to better tolerate the more nutrient-stressed conditions grew larger and also had higher lipid content.

Absolute Consumption

Fresh weight consumption increased significantly as the diets were diluted (Fig. 4-2A). At the 10% nutrient level, fw consumption increased from 2.2 to 2.5-fold compared with the undiluted diet and 1.5 to 1.7-fold compared with the 19% nutrient level diets. Additionally, fw consumption of the 19% nutrient level diets was 1.4 to 1.5-fold greater than that of the undiluted diet. However, fw consumption within each nutrient level did not differ significantly.

Fig. 4-1. Changes in lipid content (% dw) for S. frugiperda pupae reared as larvae on diets of different nutrient levels obtained through dilution with cellulose and water. Means within the 10 or 19% nutrient levels with the same letter are not significantly different according to a Tukey-Kramer ($P=0.05$) procedure and a Student's t test, respectively.



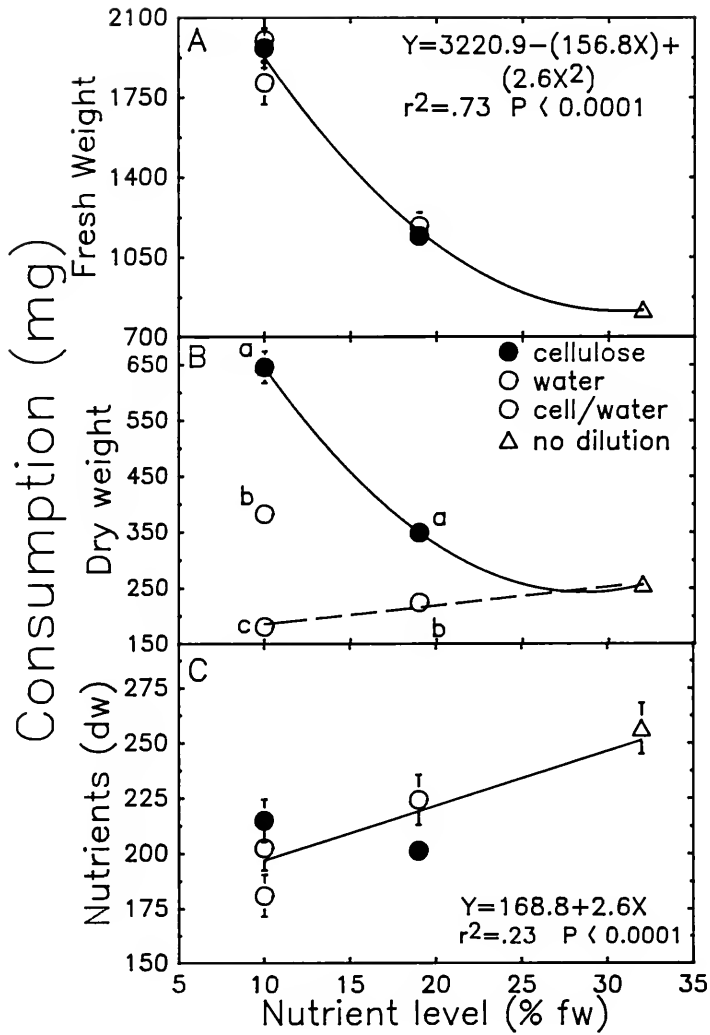
This increase in fw consumption was not sufficient to offset the dilution of nutrients in the water-diluted diets (# 4 and 6). Consequently, dw intake decreased significantly with increased dietary water content (i.e., decreased nutrient level; Fig. 4-2B). However, diets diluted with cellulose (# 2, 3 and 5) had the opposite effect; dw consumption increased significantly with cellulose dilution of diet 1. The dw consumption of the diet diluted with both cellulose and water (# 5) was intermediate between the entirely water- and entirely cellulose-diluted diets.

Although dw consumption was much greater on the cellulose-diluted diets compared with the water-diluted diets, when cellulose was subtracted from the dw values to calculate 'nutrient' consumption, these values were more similar between the water- and cellulose-diluted diets at each nutrient level (Fig. 4-2C). Nutrient intake declined significantly as nutrient level was reduced through either water or cellulose dilution. These data suggest that in response to dilution with either diluent, caterpillars increased their absolute fw consumption but they were unsuccessful in maintaining nutrient intake compared to that on the undiluted diet.

Relative Consumption

The relative rate of fw consumption [$RCR_{FW} = fw$ consumption (mg)/(average caterpillar dw (mg)/developmental

Fig. 4-2. Changes in absolute fw, dw and nutrient (i.e., cellulose-free dw) consumption by S. frugiperda larvae reared on diets of different nutrient levels obtained through dilution with cellulose and water. Means within the 10 or 19% nutrient levels with the same letter are not significantly different according to a Tukey-Kramer ($P=0.05$) procedure and a Student's t test, respectively. When letters are lacking, differences within nutrient levels were not significant. Solid line in B: $Y=1198.8-(67.1X)+(1.2X^2)$; $r^2=.89$; $P < 0.0001$. Dashed line in B: $Y=151.8-(3.4X)$; $r^2=.37$; $P < 0.0001$.

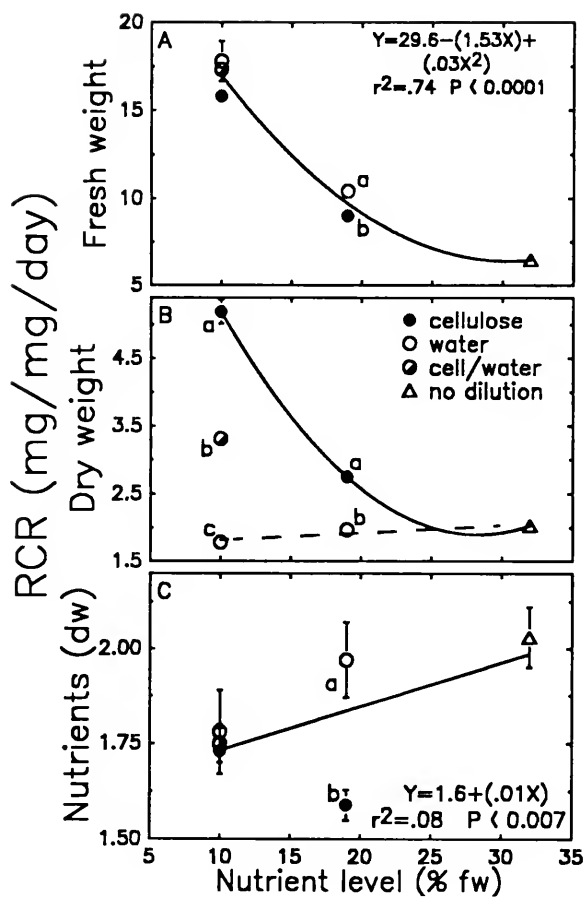


time (d)] increased significantly with diet-dilution (Fig. 4-3A), similar to absolute consumption (Fig. 4-2A). At the 10% nutrient level, the diluent did not affect RCRFW, but at the 19% nutrient level, RCRFW was significantly higher on the water-diluted diet (# 4) than on the diet diluted with cellulose (# 2) ($P < 0.02$, $t=2.51$, $df=27$).

Similar to absolute dw consumption, the relative rate of dw consumption [$RCRDW = \text{dw consumption (mg)} / \text{average caterpillar dw (mg)} / \text{developmental time (d)}$] was greatly influenced by the diluent (Fig. 4-3B). The highest RCRDW was found on the diet containing the greatest cellulose content (# 3), while the diets devoid of added cellulose had the lowest RCRDW. Diets containing intermediate levels of added cellulose (# 2 and 5) had intermediate RCRDW values. Unlike absolute dw consumption, RCRDW did not change significantly with water dilution (diets # 1, 4 and 6; $r^2=.07$; $P=0.08$). In other words, although caterpillars on the water-diluted diets ate less dw (Fig. 4-2B), the decrease was proportionate to their reduced biomass gain. Thus, relative to caterpillar biomass, increased fw consumption on the water-diluted diets stabilized the rate of dw intake.

The relative nutrient consumption rate [$RCRNU = \text{dw consumption (cellulose-free, mg)} / \text{average caterpillar dw (mg)} / \text{developmental time (d)}$], similar to absolute nutrient intake (Fig. 4-2C), declined with diet dilution (Fig. 4-3C).

Fig. 4-3. Changes in relative fw, dw, and nutrient (cellulose-free dw) consumption rate [RCR=mg consumed/mean body dw (mg)/day] by *S. frugiperda* larvae reared on diets of different nutrient levels obtained through dilution with cellulose and water. Means within the 10 or 19% nutrient levels with the same letter are not significantly different according to a Tukey-Kramer ($P=0.05$) procedure and a Student's t test, respectively. When letters are lacking, differences within nutrient levels were not significant. Solid line in B: $Y=9.8-(0.6X)+(0.01X^2)$; $r^2=.92$; $P < 0.0001$. Dashed line in B merely connects the water-diluted diets with the undiluted diet; the regression equation is not significant.



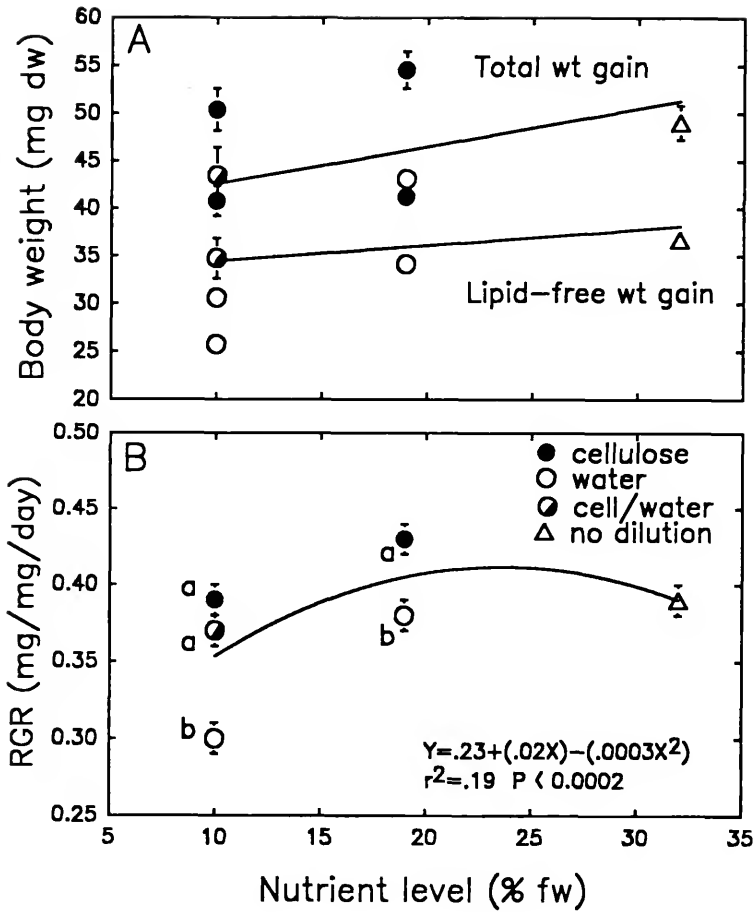
The RCRNU on the water-diluted diet (# 4) was significantly greater (1.2-fold) than that on the cellulose-diluted diet (# 2) at the same (19%) nutrient level ($P < 0.001$, $t=3.67$, $df=27$). This difference in RCRNU on these two diets occurred because caterpillars on diet 4 had smaller average biomass (10% less) but ate 11% more nutrients (although not significantly different; Fig. 4-2C) compared with those on diet 2. No differences were detected in the RCRNU among the treatments at the 10% nutrient level.

Growth and Development

Final pupal dw changed little with dietary dilution, except when the diluent consisted of water. The final dw of pupae from the 10% nutrient level diet diluted entirely with water (# 6) was ca. 40% less than that of pupae from the diet producing the heaviest pupae (# 2) and was also significantly less than the other treatments at the 10% nutrient level (# 3 and 5; Table 4-2). Similarly, at the 19% level caterpillars reared on the wetter diet (# 4) weighed significantly less than those from the comparable drier diet (# 2).

Total and lipid-free caterpillar biomass gain (dw) decreased with dietary dilution (Fig. 4-4A). The slope was significantly steeper for total dw gain (0.45 ± 0.14 ; $P < 0.001$, $t=3.37$, $df=164$) than that of lipid-free dw gain (0.21 ± 0.10), indicating that pupal lipid accounted for a greater proportion of the total biomass gain as nutrient level

Fig. 4-4. Changes in (A) body weight (total and lipid-free) and (B) relative growth rate [RGR=dw gain (mg)/mean body dw (mg)/day] for *S. frugiperda* larvae reared on diets of different nutrient levels obtained through dilution with cellulose and water. Means in the lower panel (B) within the 10 or 19% nutrient levels with the same letter are not significantly different according to a Tukey-Kramer ($P=0.05$) procedure and a Student's t test, respectively. Total weight gain: $Y=37.3-(.45X)$; $r^2=.12$; $P=0.002$. Lipid-free weight gain: $Y=31.7-(0.21X)$; $r^2=.05$; $P=0.03$.



increased; this is consistent with the decreases in lipid content (% dw) on lower nutrient level diets (Fig. 4-1).

Relative growth rate [$RGR = \text{biomass dw gain (mg)} / \text{average caterpillar dw (mg)} / \text{developmental time (d)}$] decreased with dietary dilution below the 19% nutrient level, and water-diluted diets reduced RGR to a greater extent than did the cellulose treatments (Fig. 4-4B). At the 19% nutrient level the RGR on diet 4 (water dilution) was significantly less than that on diet 2 (cellulose dilution; $P < 0.002$, $t = 3.47$, $df = 27$) and at the 10% nutrient level, RGR on diet 6 (water dilution) was significantly less than diets 3 (cellulose dilution) and 5 (water and cellulose dilution).

Feeding Efficiencies

Leaf-feeding lepidopteran larvae are not known to digest cellulose (Martin 1983); thus, the efficiency of digestion and absorption of dw diet [$ADDW = 100 * (\text{ingestion-feces}) / \text{ingestion (all dw, mg)}$], which estimates the proportion of ingested food (dw) that is digested and absorbed, decreased on those diets (# 2, 3 and 5) containing added cellulose (Table 4-2). Higher ADDW values occurred on the diets with highest water contents (81 and 90% fw) lacking added cellulose (# 4 and 6, respectively).

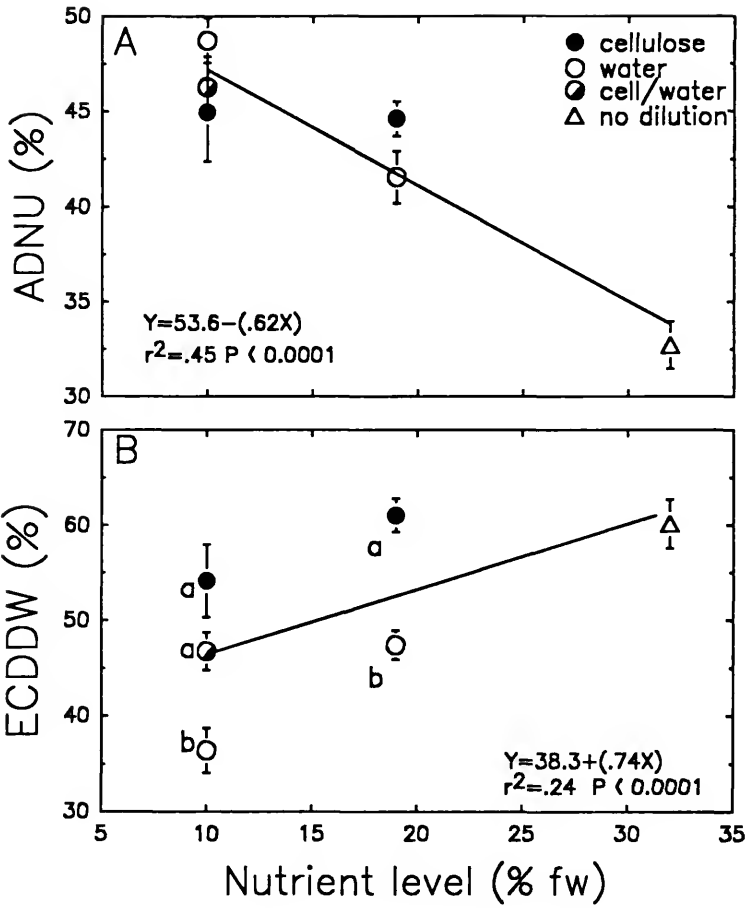
The efficiency of digestion and absorption of nutrients [$ADNU = 100 * (\text{ingestion-feces}) / \text{ingestion (all cellulose-free, dw, mg)}$] increased with diet dilution (Fig. 4-5A). No

differences were found in the ADNU among the different dilution treatments at the 10 or 19% nutrient levels; thus, increases in either dietary diluent equally increased the ADNU value. Despite the significant linear regression, increased cellulose dilution (# 2; closed circle at the 19 nutrient level) to the 10% nutrient level (# 3; closed circle) did not result in a further increase in ADNU.

The efficiency of conversion of digested food into insect tissue [$ECDDW = 100 \times \text{biomass gain} / (\text{ingestion} - \text{feces})$ (all dw, mg)] is based only on dw digestible material; water and cellulose are excluded from its calculation. Therefore, ECDDW is equivalent to ECDNU and I will refer to ECDDW hereafter. The ECDDW decreased with dietary dilution and was affected differently depending upon the diluent (Fig. 4-5B). The cellulose-diluted diet at the 19% nutrient level (# 2) had a higher ECDDW ($P < 0.0001$, $t=5.83$, $df=27$) than the water-diluted diet (# 4), as did the cellulose-diluted diets at the 10% nutrient level (# 3 and 5) relative to the comparable water-diluted diet (# 6).

The efficiency of converting ingested food into insect tissues [$ECIDW = 100 \times \text{biomass gain} / \text{ingestion}$ (all dw, mg)], which is the product of ADDW and ECDDW, decreased on the cellulose-diluted diets (# 2, 3 and 5; Table 4-2). When cellulose was removed from the calculations, the efficiency of conversion of ingested nutrients (ECINU; which is the product of ADNU and ECDDW) increased with cellulose-dilution

Fig. 4-5. Changes in digestion and absorption of nutrients [$ADNU=100 \times (\text{ingestion} - \text{feces}) / \text{ingestion}$ (all dw, cellulose-free, mg)] and efficiency of converting digested food to insect tissue [$ECDDW=100 \times \text{dw gain} / (\text{ingestion} - \text{feces})$ (all dw, cellulose-free, mg)] by *S. frugiperda* larvae reared on diets of different nutrient levels obtained through dilution with cellulose and water. Means within the 10 or 19% nutrient levels with the same letter are not significantly different according to a Tukey-Kramer ($P=0.05$) procedure and a Student's t test, respectively. When letters are lacking (upper panel; A), differences within nutrient levels were not significant.



relative to the undiluted and water-diluted diets (Table 4-2). This occurred because of the combination of relatively high ADNU and ECDDW on the diets diluted entirely with cellulose. The ECINU for diet # 5 was intermediate between the water- and cellulose-diluted diets. Although the ADNU was high on diet 6, this was insufficient to offset the low ECDDW resulting in a low ECINU. The reverse occurred for diet 1 where the high ECDDW was insufficient to offset the low ADNU, resulting in a lower ECINU.

Because the ingested food probably moved more rapidly through the larval guts as consumption (RCRFW) increased on the diluted diets, ADNU was expected to decrease due to shorter retention time or saturation of digestive enzymes in the midgut. However, this did not occur; RCRFW and ADNU were instead positively correlated [$r=0.56$; $P < 0.0001$; $Y=32.6+(0.83X)$]. On the other hand, RCRNU and ADNU were negatively correlated [$r=0.28$; $P < 0.01$; $Y=54.6-(6.4X)$]. Thus, these data suggest that the more rapid intake of nutrients with increased nutrient level rather than total mass (fw) of the food, with increased diet dilution, was responsible for the decline in ADNU.

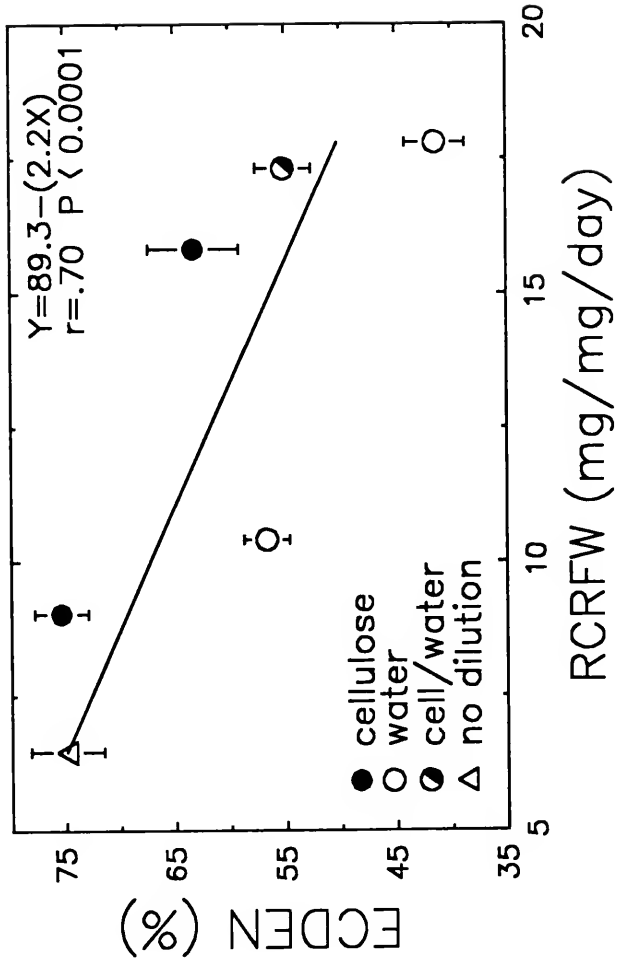
With the increased rates of fw consumption on the diluted diets, caterpillars presumably expended more energy ingesting and processing food than caterpillars feeding at lower rates. If feeding faster constitutes a substantial enough energy expenditure under these conditions to be

detectable, then the energy conversion efficiency [ECDEN=energy content of the biomass gained (J)/(absorbed energy (J))] should decline as RCRFW increased. As RCRFW increased, ECDEN decreased significantly (Fig. 4-6), supporting this hypothesis.

Discussion

My data demonstrate the compensatory feeding ability of S. frugiperda in response to diets diluted with cellulose or water. Because of the increased fw consumption, pupal dw on the cellulose-diluted diets was equivalent to that on the undiluted diet. Final pupal weight on the moderately water-diluted diet was also similar to that on the undiluted diet (although significantly lower than that on diet 2); however, pupal weight on the highest water dilution diet was less than all other treatments. Changes in feeding were also reported for other Spodoptera spp. fed silica- or cellulose-diluted artificial diets (Peterson et al. 1988), adulterated or imbalanced artificial diets (Johnson and Bentley 1988; Simpson et al. 1988), fertilized host plants (Al-Zubaidi 1983; but see Manuwoto and Scriber 1985a; 1985b) and different host plant species (Soo Hoo and Fraenkel 1966; Scriber 1979). Additionally, these results for S. frugiperda are similar to the compensatory feeding responses of caterpillars of another noctuid (Anticarsia gemmatilis)

Fig. 4-6. Changes in the efficiency of converting digested energy $[ECDEN=100 \cdot dw \text{ gained/absorption, all J}]$ with relative consumption rate fw (RCRFW; see Fig. 4-3 for formula) by S. frugiperda larvae reared on diets of different nutrient levels obtained through dilution with cellulose and water.



fed similarly diluted diets (Slansky and Wheeler 1989; in press).

For the most part, the results of other studies evaluating the feeding responses of S. frugiperda were similar to those found here. Crocomo and Parra (1985) demonstrated apparent compensation for variable diets by S. frugiperda caterpillars that achieved the same RGR on maize, wheat or sorghum. Differences in performance included increased consumption rates but poor absorption and conversion of corn, decreased consumption rates but greater absorption and conversion of wheat, and moderate consumption rates and conversion of sorghum. The forage quality of different bermudagrass varieties influenced S. frugiperda caterpillar feeding and food utilization (Quisenberry and Wilson 1985; Lynch et al. 1986; Chang et al. 1987; Jamjanya and Quisenberry 1988). Varieties with higher levels of crude protein and higher digestibility (based on ruminant digestibility) improved caterpillar survival, reduced developmental time and increased fw gain (Lynch et al. 1986).

Although many of the values for nutritional indices presented in the remaining studies are different from my data and those of Crocomo and Parra (1985), within-study comparisons suggest that consumption increases, and growth rate and food utilization efficiencies decrease on poorer quality forage (Quisenberry and Wilson 1985; Jamjanya and

Quisenberry 1988). I conducted stepwise multiple regressions on the means published by these authors and found that some of the changes in caterpillar performance on foliage were similar to those that I found using artificial diets (Table 4-3), including decreased dw consumption, growth, and biomass gain with increased foliage water content. However, contrary to my results, conversion efficiencies (ECDDW and ECIDW) increased and ADDW decreased with greater foliar water content (although ECIDW decreased with higher water content foliage according to Jamjanya and Quisenberry 1988). These discrepancies are presently unexplained but they may be caused by simultaneous changes and interactions among nutrients, water, and allelochemicals occurring in different host plant species and varieties (e.g., Scriber 1977; 1979). My experimental design allowed me to make comparisons among different levels of nutrition, water and cellulose with a presumed minimum of interactive influences.

Dilution of the diets with cellulose increased the rates of fw and dw intake, and reduced the efficiency of digestion and absorption (ADDW) due to the indigestibility of cellulose (Martin 1983). Similar responses to cellulose were reported for A. gemmatalis (Slansky and Wheeler in press) and S. eridania (Peterson et al. 1988). However, the large increase in ADNU with cellulose dilution was unique to the present study.

Table 4-3. Results of stepwise multiple regressions relating various measures of *Spodoptera frugiperda* caterpillar post-ingestive performance to different components of forage quality. Analyses included (A) the published means from Quisenberry & Wilson (1985) and (B) indices calculated from data presented in Jamjanya & Quisenberry (1988), however only the significant relationships ($P < 0.05$) are listed.

Regression Equation:			
Performance ¹ measure	Forage ² quality	r ²	P
A) RGR=	2.0 - 0.02 (IVDDM)	0.62	0.035
DWCONS=	975.6 - 9.5 (WATER)	0.81	0.006
ADDW=	149.6 - 1.5 (WATER)	0.72	0.02
ECIDW=	-9.7 + 0.4 (WATER)	0.57	0.05
ECDDW=	-91.8 + 1.9 (WATER)	0.81	0.006
B) RGR=	0.98 - 0.01 (WATER)	0.59	0.02
WTGN=	73.7 - 0.74 (WATER)	0.63	0.01
FWCONS=	-1696.4 + 35.3 (WATER)	0.74	0.003
ECDDW=	-1.5 + 6.06 (AIL)	0.57	0.02
ECIDW=	33.1 - 0.34 (WATER)	0.53	0.03

¹ Caterpillar performance indices (dw unless otherwise noted) refer to: RGR=relative growth rate (weight gain/mean weight/day); DWCONS=dry weight consumption; ADDW=approximate digestibility (ingestion-feces/ingestion); ECIDW=efficiency of conversion of ingested food (weight gain/ingestion); ECDDW=efficiency of conversion of digested food (weight gain/ingestion-feces); WTGN=weight gain; & FWCONS=fresh weight consumed.

² The forage quality indices refer to: IVDDM=in vitro digestible dry mater; and AIL=acid insoluble lignin.

Water Regulation

Water-diluted diets reduced caterpillar performance more than an equivalent dilution with cellulose. Despite increased RCRNU on the water-diluted diet (# 4) at the 19% nutrient level compared with the cellulose-diluted diet (# 2), RGR and lipid content were higher on the latter. This is explained by significantly higher nutrient conversion efficiencies (ECINU and ECDDW) on diet 2 than on diet 4. Additionally, despite the increased fw consumption on the most diluted diets (# 3, 5 and 6; all 10% nutrient level), caterpillars on the highest water content diet (# 6) had a greater developmental time, and decreased RGR and weight gain compared with those on the other two diets. Pupal lipid content decreased on all treatments at this nutrient level regardless of the diluent, but the decrease was greatest on the diet with the highest water content (# 6). These data suggest that, compared with cellulose dilution, the processing of high water diets constitutes a significantly greater cost (lower ECINU and ECDDW) to these caterpillars despite the higher efficiency of nutrient digestion and absorption.

Although dietary water level ranged from 68 to 90% fw, the pupal water content varied little, suggesting that a relatively constant water balance was actively maintained. When another noctuid (*A. gemmatalis*) was reared on a high water diet (89% fw), pupal lipid content, caterpillar growth

and food conversion efficiencies were significantly reduced (Slansky and Wheeler 1989). The diets in the previous and the present studies contained both less and greater water content than the caterpillars. On the high water diet A. gemmatalis exhibited high mortality (though not significant, a 2-fold increase over the driest diet), a 12 percentage point increase in pupal moisture, a 46% reduction in dw gain and a 73% reduction in ECDDW. However, performance of S. frugiperda was less severely affected on the wetter diet, with no significant mortality, only a 5 percentage point increase in pupal moisture, a 37% reduction in dw gain and a 62% reduction in ECDDW. Thus, although metabolic costs may have been high for both species, as indicated by reduced ECDDW, S. frugiperda was better able to regulate body moisture, and this was apparently beneficial in terms of improved growth and survival. Additional evidence for noctuid caterpillars maintaining water balance was presented by Reese and Beck (1978); the water content of Agrotis ipsilon (Hufnagel) caterpillars varied little over a range of dietary water similar to that used in my study. Furthermore, the water content of various caterpillar tissues (e.g., foregut, midgut, ileum, carcass) of Manduca sexta (L.) varied much less than dietary moisture (Reynolds and Bellward 1989).

Regulation of Consumption and Digestion

Very little information is available describing the regulation of feeding and digestion of lepidopterous larvae (Chapman 1985; Simpson and Simpson in press). The available data suggest that both activities are mediated by juvenile hormone (Muraleedharan and Prabhu 1981; Chapman 1985). Meal size may be regulated by a nutrient feedback mechanism, as found with M. sexta (Timmins and Reynolds, in prep.) or by volumetric feedback from midgut stretch receptors (see Bernays 1985 for a review), as demonstrated in the acridids Locusta (Simpson 1983) and Schistocerca (Roessingh and Simpson 1984). Ingestion may also activate digestive enzymes by a secretagogue mechanism (Chapman 1985).

Regardless of the diluent used in my study, RCRFW increased in a graded response to increased levels of dilution. These results support the conclusion of Timmins and Reynolds (in prep.) that consumption by M. sexta caterpillars is regulated by nutrient feedback and not volumetric feedback from the midgut. As the S. frugiperda larvae were more nutrient stressed on the diluted diets, they responded by increasing their rate of fw intake.

Relative consumption rate of nutrients (RCRNU) increased with nutrient level, especially at the 19% nutrient level on the water- (# 4) compared with cellulose-diluted diet (# 2). These results suggest that the factors regulating RCRNU at this nutrient level were influenced

differently by the type of diluent. Water may have been removed from the ingested food as it passed through the foregut (Timmins et al. 1988, citing unpublished data from Reynolds and Bellward), producing two effects that further modified the regulation of the rate of nutrient intake. First, as water was removed from the food (perhaps from both the fore- and midgut) this probably reduced hemolymph osmolality, stimulating the consumption of more frequent meals (Abisgold and Simpson 1987) by the nutrient feedback mechanism (Timmins and Reynolds, in prep.). Second, the removal of water from the food may allow greater nutrient consumption before stimulation of the hypothesized volumetric midgut stretch receptors. Diet # 2 probably could not be concentrated to the same extent prior to passage to the midgut, and higher hemolymph osmolality or more rapid stimulation of stretch receptors ensued, reducing meal size. Similarly, RCRNU of A. gemmatalis caterpillar was significantly higher on the water-diluted compared with the cellulose-diluted diet at the same (19% fw) nutrient level (Slansky and Wheeler in press). Cautious interpretations should be made when comparing ingestion of plant material and artificial diets; however, a similar mechanism may have extended consumption bouts and overall greater fw consumption for M. sexta on the wetter tobacco leaves (85% fw) compared with artificial diet (77% fw) (Reynolds et al. 1986). Although nutrient feedback is most

important in the regulation of meal size in M. sexta caterpillars compared with volumetric feedback (Timmins and Reynolds, in prep.), the latter influence may nonetheless occur in other lepidopterous species.

No differences in RCRNU were observed among the diluents at the 10% nutrient level, suggesting that consumption may be regulated differently during conditions of extreme nutrient stress. At this nutrient level other regulation mechanisms may be overridden by the nutrient feedback stimulus. Such a mechanism would be adaptive under conditions where nutrients fall below some critical level that threatens survival of the developing caterpillars.

In the present study, ADNU increased as much as 1.5-fold with diet dilution but was not affected significantly by the type of dietary diluent. Because there were no differences among the dilution treatments at either the 10 or 19% nutrient levels, digestion and absorption apparently increased as a result of ingesting either greater volumes of food or more diluted food. Similarly, A. gemmatalis digestion and absorption (ADNU) increased as much as 1.3-fold over a similar range of water dilution (Slansky and Wheeler 1989). However, A. gemmatalis ADNU did not change significantly as it did in the present study when the diets were diluted as in diets 4 and 5 (Slansky and Wheeler in press). Additional midgut digestive enzymes may have been secreted by S. frugiperda with increased fw consumption,

resulting in increased ADNU. Alternatively, caterpillars may have digested and absorbed a reduced proportion of the more concentrated nutrients on the less diluted diets, contributing to their decreased ADNU.

Energy Cost of Consumption

The costs of food consumption and utilization by caterpillars have been estimated to be minimal (ca. 3%) relative to the energy content of the consumed food (Aidley 1976; McEvoy 1984). Despite an increase in consumption from 0 to 8 mg h⁻¹ on different plant parts by cinnabar moth caterpillars [*Tyria jacobaeae* (L.)], oxygen consumption increased only 1.8-fold (McEvoy 1984). In the present study, the cost of feeding may be more important, because not only did the energy content (J mg⁻¹ dw) of the food decline with dilution by indigestible material, but also RCRNU (and thus the relative consumption rate of energy) declined with diet dilution (although this was moderated by increased ADNU). Increased feeding (2.4-fold fw, 2.5-fold dw) on diets with less energy content (31% of undiluted diet) may constitute a significant reduction in net energy gain. Assuming the undiluted diet consisted of 18,410 mJ mg⁻¹ dw and the energy expended eating this diet was 468.5 mJ mg⁻¹ of food (dw) (values from McEvoy 1984), and applying the regression equation of McEvoy (1984) describing changes in respiration with increased ingestion (dw) to my data for

the highest rates of dw consumption on the 22% fw cellulose-diluted diet (# 3), respiration should have increased $51.1 \mu\text{l O}_2 \text{ mg}^{-1}$ food ingested over the calculated rate on the undiluted diet (286.5 and $235.4 \mu\text{l O}_2 \text{ mg}^{-1}$, respectively). Assuming each $\mu\text{l O}_2$ respired corresponds to 20.5 mJ (Aidley 1976), I calculate the additional energy expended ($1047.6 \text{ mJ mg}^{-1}$) in consuming the highest cellulose-diluted diet (containing $5707.1 \text{ mJ mg}^{-1}$) may have constituted 18% of the energy content of the ingested food. This value is considerably higher than that reported by McEvoy (1984) and Aidley (1976) on undiluted food. Further evidence for this increased energy expenditure may have been manifested in reduced energy conversion efficiency (ECDEN) (ca. 15% less on diet 3) at the higher RCRFW levels. However other costs, such as uric acid production and water resorption on drier diets (65% water) may reduce ECDEN (Van't Hof and Martin 1989) as may processing wetter diets (see previous discussion). These results suggest that foliage feeding caterpillars must select host plant of adequate nutritional and energetic content to avoid high consumption and increased energy costs.

CHAPTER 5
COMPENSATORY DILUTION-INDUCED FEEDING
INCREASES BY ANTICARSIA GEMMATALIS LARVAE
WHEN FED SOYBEAN ALLELOCHEMICALS

Compensatory Feeding Responses in Larval Herbivores

Compensatory feeding increases were demonstrated in the previous chapter (Chapter 4) in fall armyworm larvae and elsewhere with the velvetbean caterpillar (Slansky and Wheeler 1989). These data suggest compensatory feeding increases may be a generalized response exhibited by noctuid larvae to acquire sufficient nutrients when diets are diluted with indigestible material (e.g., cellulose) or water.

The consequences of such increased fresh weight (fw) consumption have not been addressed, yet preliminary data (Slansky and Wheeler in prep.) suggest that the velvetbean caterpillar may not be able to perceive or respond adaptively when confronted with both potentially toxic levels of dietary allelochemicals and diets diluted with water. In this preliminary study, velvetbean caterpillar larvae fed water diluted diets that incorporated the methylxanthine alkaloid, caffeine, at sublethal concentrations increased their fw consumption on the diluted diets and thereby ingested an effective dose, reducing

survival. However, as the velvetbean caterpillar is not known to feed on plant species that contain caffeine, there is no reason to believe that the species would recognize the potential toxin and respond adaptively. Because the velvetbean caterpillar is a common soybean pest, the species may recognize soybean allelochemicals and respond adaptively when fed an effective dose incorporated in diluted artificial diets. Thus, I hypothesize here that velvetbean caterpillar larval compensatory feeding increases will be moderated when fed diluted diets containing sublethal doses of potentially toxic soybean allelochemicals.

Methods and Materials

Preliminary Study

The initial step in this study involved the determination of the benzene fraction extract dose that would reduce velvetbean caterpillar relative growth rate (RGR) or survival by ca. 50% and the change in RCRFW when fed diets of different levels of dilution. With this information, I could then feed larvae a combination sublethal dose of the benzene extract fraction while diluting the diet, such that increased fw consumption of the diluted diet may entail ingestion of a lethal (or effective) dose of benzene extract fraction.

Diet preparation and rearing conditions were as described in Chapter 4. Diet formulations consisted of the

standard artificial diet (Greene et al. 1978) incorporating different amounts (0, 0.19, 0.25, 0.31, 0.38 and 0.44% dw) of the benzene extract fraction from mite-free plants (Bragg, from Chapter 3). Velvetbean caterpillar larvae were reared (15 replicates) on diets from the third instar through to pupation. Larval RGR values and percent mortality to prepupae were calculated for each treatment as described previously (Chapter 4). Larval mortality (to the prepupal stadium) data were analyzed by a G-test (Zar 1984) and the LD_{50} value was calculated with a probit analysis (Finney 1971) calculated with SAS/PC (SAS Institute Inc. 1987).

Results reported elsewhere (Slansky and Wheeler 1989) were used to establish the degree of increased compensatory feeding by the velvetbean caterpillar larvae when fed water-diluted diets. These results indicated that velvetbean caterpillar larvae increased absolute fw consumption 1.2- to nearly 2-fold on diets composed of 86-89% water (comparable to the 14-11% nutrient levels of Chapter 4) compared with the control larvae feeding on diet composed of 65% water. Calculations of these data indicated that velvetbean caterpillar larvae would increase their dietary fw relative consumption rate (RCRFW) from 6.5, to 10.5 and 16.6 mg/mg/day on diets diluted with 65, 79 and 89% fw water, respectively.

Determination of Compensatory Ability When Fed Diluted Diet
Containing Sublethal Levels of Soybean Foliar
Extractables

Using the preliminary results from the above studies, third instar velvetbean caterpillar larvae were fed the standard artificial diet (65% fw) diluted with water to 79 and 89% fw incorporated with sublethal doses of soybean foliar extractables (benzene fraction). If the fw ingestion increased as found previously on diluted diets, the intake of any allelochemical added to the diet would increase. In combination with the extra water content, the diets were formulated to contain concentrations of benzene fraction extract that would maintain a constant ingestion of allelochemical as the caterpillars increased fw consumption on the diluted diets. A range of doses were included that approximated three effective doses (achieved as fw consumption increased on diluted diets). These doses would reduce survival 24, 49 and 74% compared with the control diets (i.e., LD_{24} , LD_{49} and LD_{74}). Nutritional indices were calculated by a gravimetric technique (Waldbauer 1968, Slansky and Scriber 1985); these included larval fw relative consumption rate (RCRFW), absolute consumption of the benzene fraction extract and RGR. Larval mortality (to the prepupal stadium) data were analyzed by hand with a G-test (Zar 1984). The remaining data (randomized complete block experimental design) were analyzed by an analysis of variance (ANOVA) with means separated by a Tukey-Kramer test

and simple linear regression (Sokal and Rohlf 1981) using SAS/PC (SAS Institute Inc. 1987). Linear regression slopes were compared by hand with a Student's t -test (Zar 1984).

Results

Preliminary Study

High larval mortality occurred on diets with extract concentrations greater than 0.29% dw (Table 5-1). Analysis of the non-zero data (i.e., excluding the 0.56% concentration level at which all larvae died) indicated significant differences occurred in mortality among the concentration levels (Table 5-1). Additionally, probit analysis of the allelochemical treatment data (i.e., excluding the control) indicated that the $LD_{50}=0.38\%$ dw (upper and lower fiducial limits=0.64 and 0.30, $\chi^2=14.1$, $df=4$, $P=0.0071$).

Table 5-1. Mortality (to the prepupae stadium) and RGR (15 replicates) from a preliminary study determining the level of activity in various concentrations of the benzene extract fraction (from mite-free Bragg) incorporated in artificial diet and fed to third instar velvetbean caterpillar larvae.

Conc ¹	RGR ²	(±se)	% Mortality ³
0	0.36	(±0.02)	6.7
0.17	0.36	(±0.02)	6.7
0.23	0.38	(±0.01)	6.7
0.29	0.34	(±0.02)	6.7
0.34	0.34	(±0.02)	33.3
0.40	0.29	(±0.02)	53.3
0.56	-	-	100.0

¹ Concentration expressed as percent dry weight of diet.

² RGR=relative growth rate [biomass gain (mg)/average dry weight (mg)/day]. RGR differences among concentration levels were not significantly different according to a Tukey-Kramer test ($P=0.07$).

³ Percent mortality data were significantly different among concentration levels (where mortality was < 100%) according to a G-test ($\chi^2=34.6$, $df=5$ $P<0.005$).

Determination of Compensatory Ability When Fed Diluted Diet Containing Sublethal Levels of Soybean Foliar Extractables

Diets. Percent water content differed significantly for the diet classes (65%, 79% and 89% fw, Table 5-2). The diet containing the combination water content 79% fw and the LD₇₄ had a significantly lower percent water than the other 79% fw diets and yet a greater water content than the 65% fw diets. As reported previously (Chapter 4), slight variations in weighing dietary ingredients and their storage and dispensing may account for these variations. Although

statistically significant, they are assumed to have little biological relevance as 1-2 percentage points (fw) does not significantly influence larval performance (Slansky and Wheeler 1989).

Larval mortality. Larval mortality was relatively low on the control treatments at all water content levels and increased to 100% on all dilution levels of the LD₇₄ treatment (Table 5-2). Mortality was greater than 50% on the LD₄₉ diets. Due to the large number of treatment combinations with either 0 or 100% mortality, the data were analyzed by dietary water content (irrespective of effective dose), and effective dose (irrespective of water content); both effects were significant.

Larval Consumption

As demonstrated in Chapter 4 (for the fall armyworm), RCRFW (mg fw consumed/mg mean body dw/day) of velvetbean caterpillar larvae increased significantly on the more water-diluted diets (Fig. 5-1). The RCRFW of larvae fed the extract-free diets (control) increased 1.5- to 2.7-fold compared with the undiluted control diet. Larvae feeding on the LD₂₄ and LD₄₉ water-diluted diets increased their RCRFW at the 79% water level 1.5- and 1.3-fold and at the 89% water level 1.8- and 2.4-fold, respectively. Significant

Table 5-2. Percent water content of diets (10 replicates) and mortality of velvetbean caterpillar larvae (15 replicates) fed these diets containing three effective dose levels of the benzene extract fraction (from mite-free Bragg).

Water ¹ LD ²		% Water (\pm se) ³	% Mortality ⁴
65	0	67.1 (\pm 0.2) a	7
65	24	66.5 (\pm 0.2) a	0
65	49	67.6 (\pm 0.1) a	60
65	74	67.5 (\pm 0.2) a	100
79	0	80.1 (\pm 0.3) b	0
79	24	79.4 (\pm 0.4) b	0
79	49	80.1 (\pm 0.5) b	53
79	74	77.9 (\pm 0.2) c	100
89	0	88.5 (\pm 0.3) d	0
89	24	88.4 (\pm 0.2) d	80
89	49	87.8 (\pm 0.2) d	87
89	74	87.9 (\pm 0.1) d	100

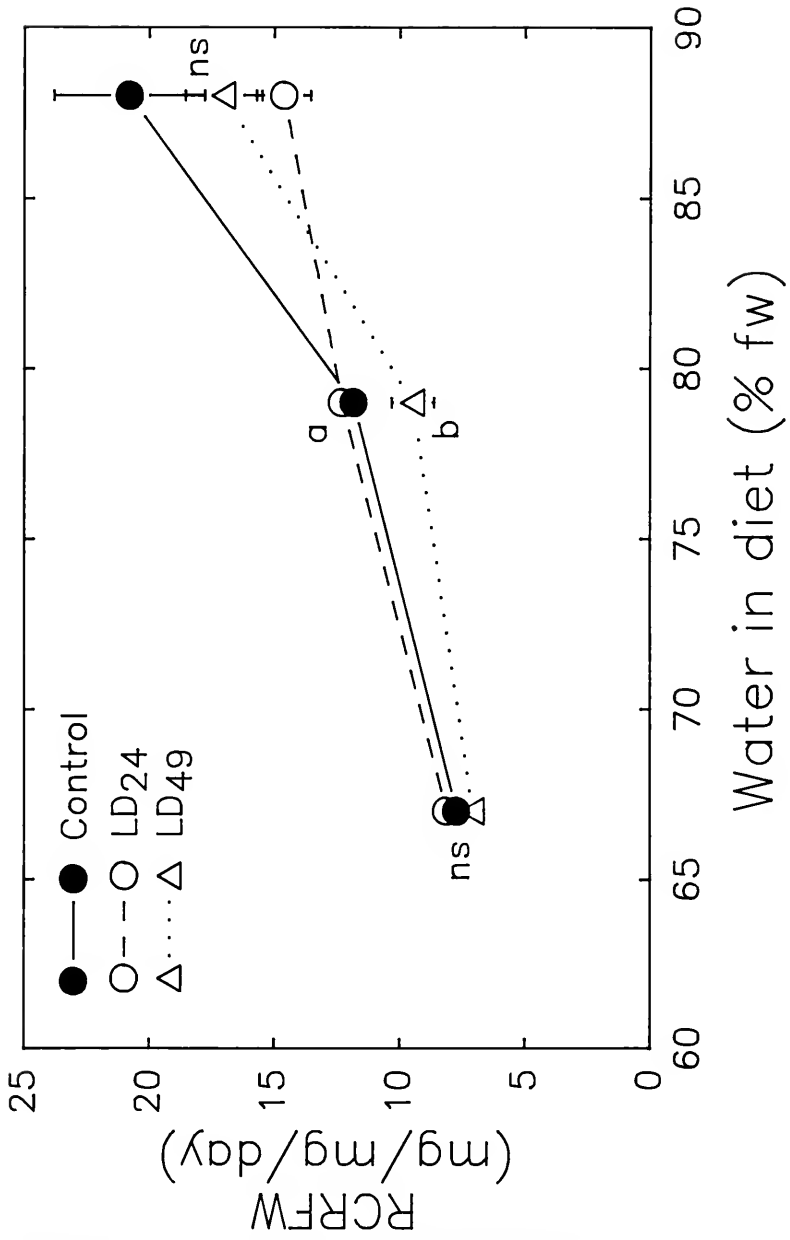
¹ Expected water content expressed as percent fresh weight of diet.

² The effective doses correspond to the LD₅₀ of 0.38% dw.

³ Means of actual percent water content followed by the same letter are not significantly different according to a Tukey-Kramer test ($P=0.05$).

⁴ Percent mortality data analyzed by water content and by effective dose, were significantly different according to a G-test ($\chi^2=99.9$, $df=2$, $P<0.005$ and $\chi^2=49.1$, $df=2$, $P<0.005$, respectively).

Fig. 5-1. Increase in relative fresh weight consumption rate (RCRFW=mg fw consumed/mg mean body dw/day) by caterpillars of the velvetbean caterpillar fed diets of different water levels and effective doses of soybean foliar extract.



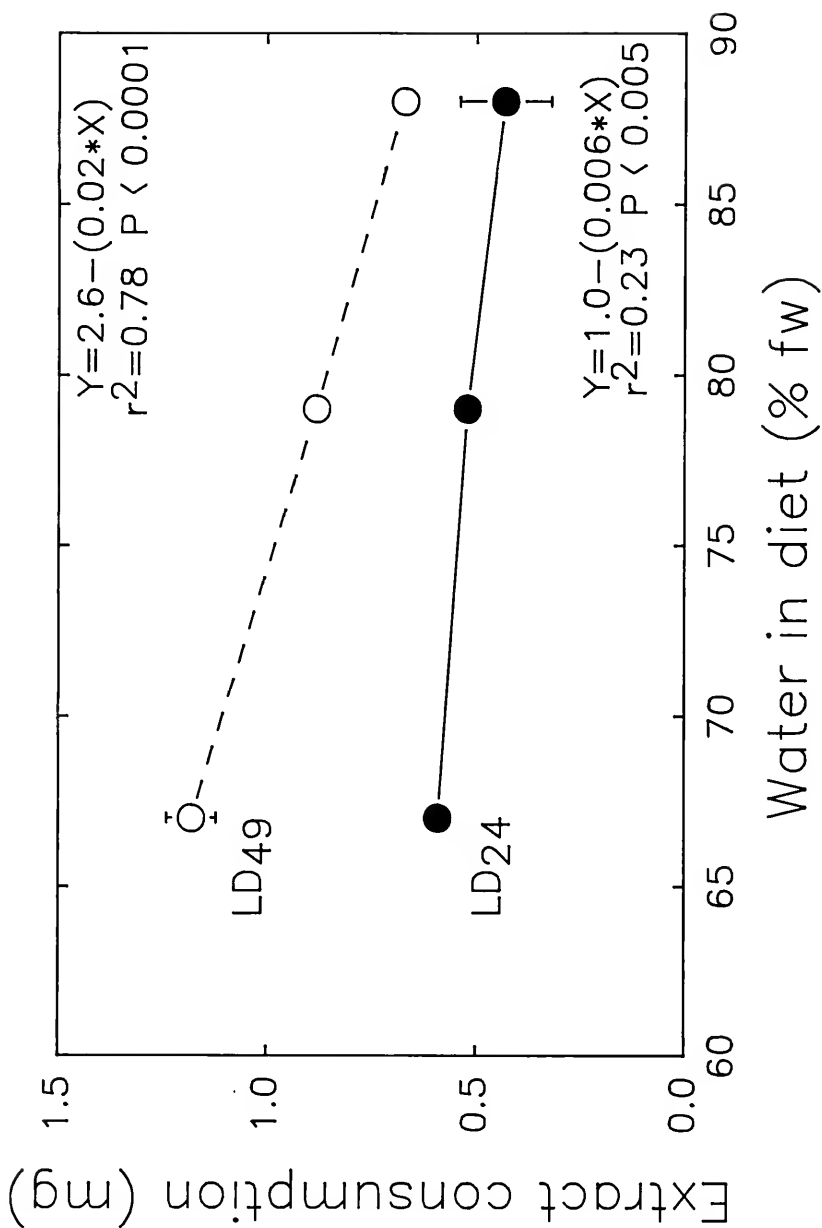
differences were found in RCRFW among the effective dose treatments at the 79% water level, where the LD_{49} RCRFW was significantly less than the other two effective dose treatments. At the 65 and 89% water levels, no significant differences were found in the RCRFW among the different effective doses ($P=0.06$ and $P=0.43$, respectively).

Although the diets were formulated to maintain the extract intake constant while RCRFW increased, the larvae did not increase RCRFW to the degree predicted on the diets containing the extract at the LD_{24} and LD_{49} levels (Fig. 5-2). The regression equations for each effective dose had significant negative slopes with increasing water dilution. Furthermore, the slope of the LD_{49} data was significantly greater than that for the LD_{24} data ($t=23.5$, $df=44$, $P<0.001$). These data suggest that larvae moderated their compensatory feeding increase when fed these toxins, and this response was most apparent on the diet containing the highest concentration of extract.

Growth and Development

The RGR data for larvae feeding on the 65 and 79% fw water content diets indicated that larvae fed the highest concentration of the extract grew significantly slower than larvae on the control or LD_{24} diets (Fig. 5-3). No significant differences were found among the effective doses

Fig. 5-2. Decrease in ingestion of soybean foliar benzene fraction extract by larvae of the velvetbean caterpillar. Artificial diets were diluted with increased levels of water and contained different effective doses of extract.



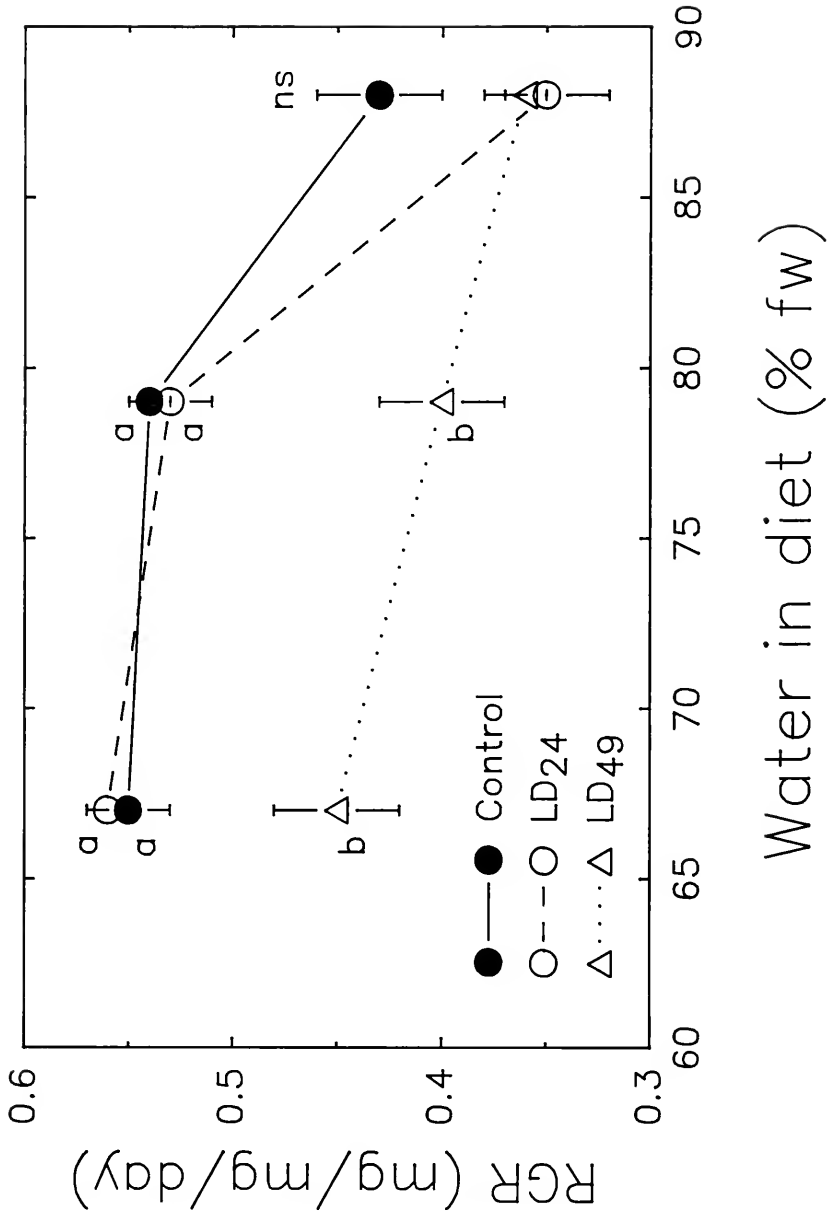
at the 89% water level ($P=0.17$). Thus, as the larvae fed on the diets containing the higher effective dose (LD_{49}) they grew slower, despite their moderated compensatory feeding response on the 79% water-diluted diet.

Discussion

The levels of mortality found in the establishment of an LD_{50} value for the benzene fraction extract were similar to the mortality data presented in Chapter 3 (Table 3-4), where 0-60% and 96-100% mortality occurred at the 0.25 and 0.50% dw concentrations of the crude fractions, respectively. However, in the present chapter, I suggest that the combined effect of dietary water dilution and extract concentration caused high mortality, resulting in low sample sizes at the 89% fw diet level. Thus, these two effects were confounded and probably precluded the detection of changes in compensatory feeding increases on the most diluted diets incorporating sublethal extract doses.

Although all larvae were from an inbred laboratory colony (ca. 96 generations), measurements of consumption and growth under high selective pressure (50-90% mortality) may not have accurately reflected the entire population. Individual variation among the larvae in their ability to tolerate the dietary toxins may have caused the differential survival. Thus, the consumption and growth data for the

Fig. 5-3. Decreases in relative growth rates (RGR=biomass gain/average dw/day) in larvae of the velvetbean caterpillar fed diets containing increased water content and different effective doses of soybean foliar extract.



survivors may reflect the values obtained for larvae more tolerant of these experimental conditions.

Compensatory feeding increases (RCRFW) occurred at both dilution levels (79 and 89% fw) on both the extract-free and the LD₂₄ and LD₄₉ treatments, and this response was comparable with the responses observed in a similar study (Slansky and Wheeler 1989). However, a significant decrease in RCRFW did occur at the 79% fw level on the LD₄₉ diet. Additionally, a decrease in extract consumption occurred on both dosage treatments (especially at the higher dose), as the diets were diluted with increasing moisture. However, decreases in RCRFW were of no apparent benefit, as RGR values decreased concurrently. Therefore, the decreases in RCRFW and extract consumption were probably due to reduced fitness of the larvae feeding on diets containing the extracts and higher water content (i.e., a result of the toxic impact of the active fraction) and not an adaptive response to reduce ingestion of potential toxins.

In conclusion, these results neither support nor refute the hypothesis that larvae moderate compensatory feeding increases when fed diets containing allelochemicals that occur in their normal host range. The high metabolic stress of larvae fed the high water content diets may have reduced the ability of larvae to respond adaptively at this dilution level. Dilution of diets with lower levels of water or similar levels of cellulose, may resolve this problem.

CHAPTER 6 GENERAL CONCLUSIONS

The potential hosts of Anticarsia gemmatalis may be traced phylogenetically through the Papilionoideae, originating from the ancestral Tephrosieae and diverging through several tribes of the Old World, New World and Temperate zones. The more advanced tribes that contained species that served as potential laboratory hosts included the New World tropical Aeschynomeneae, Old World tropical Desmodae and Phaseoleae, and the temperate Genisteae, Trifolieae and Coronilleae. No species of the African Crotalarieae served as acceptable hosts, perhaps due to the tribe's phylogenetic isolation from the more ancestral tribes or the presence of toxic allelochemicals.

A review of the allelochemical literature indicated that the isoflavones phaseol and afrormosin constitute the most active compounds discovered, causing high larval mortality (71-98%). The flavonoids in general, however, have an uncertain influence upon A. gemmatalis host range, as many may serve as either attractants or repellents. Other classes of compounds that may have an influence in restricting caterpillar host range include the pyrrolizidine, quinolizidine and erythrina alkaloids.

Benzene extractables of soybean foliage were toxic to larvae of A. gemmatalis and Spodoptera frugiperda causing reduced feeding efficiency in the former, and mortality and reduced relative growth rates (RGR) in both species. This fraction extracted from resistant soybean foliage (PI229358), also reduced the RGR values of the noctuid species Heliothis zea and H. virescens. Induced resistance factor(s) were extractable in petroleum ether causing reductions in the RGR values of third instar S. frugiperda and A. gemmatalis larvae. However, these extractables had little or no influence on mortality or the RGR values of several other noctuid larval species. Further refinement of the benzene fraction indicated that the active component(s) was acetone extractable reducing RGR values in A. gemmatalis and S. frugiperda and causing high mortality (60%) in the latter. Increased detoxication (MFO) activity in cabbage looper and fall armyworm larvae, fed diets containing these extractables, suggest that these species may tolerate plants containing these potential toxins by oxidation.

In response to diluted diets, S. frugiperda caterpillars compensated by increased fresh weight consumption rates. This compensatory response moderated the negative impact of reduced nutrient intake on the more diluted diets, such that growth (RGR) was stabilized on all but the most water-diluted diet. The assimilation of nutrients decreased on the more diluted diets, whereas the

efficiency of converting digested food to body tissue increased. However, despite the compensatory feeding, total body weight decreased on the more diluted diets, primarily because of reduced lipid content. The efficiency of converting digested energy to body tissue decreased as fresh weight consumption increased on the more diluted diets. I suggest that greater food intake constitutes a significant energy drain (increased chewing, synthesis of digestive enzymes) relative to the decreased energy content of the diluted diet. However, more direct measurements of the energy budget must be conducted to verify these data.

My data support the hypothesis that consumption rates are regulated by a nutrient feedback mechanism; additionally, this regulation may be modified by volumetric feedback from midgut stretch receptors. My data also suggest that digestive enzyme activity is correlated with consumption rates.

Velvetbean caterpillar larvae fed water-diluted artificial diets that contained sublethal doses of soybean foliar benzene extractables significantly reduced their fresh weight relative consumption rate (RCRFW) compared with larvae fed diets lacking the extract. However, larval growth (RGR) did not improve when RCRFW was decreased on the extract containing diet. Furthermore, larval mortality was high when fed diets diluted with 89% fw water. Further research is required to determine whether larvae are able to

moderate compensatory feeding increases in an adaptive manner, thereby avoiding ingestion of a lethal dose of allelochemical.

APPENDIX A

Results of mixed function oxidase detoxication activities of the larvae of two noctuid species fed artificial diets containing soybean foliar extract fractions. Cytochrome P-450 oxidase activity (pm/min/mg protein) was measured for the 10 crude midgut homogenates (Yu 1982). Each mean represents the average of two subsamples.

Frac ¹	Conc ²	Spp ³	Mean Activity	se	% of control
Control	-	VBC	154.59	2.66	100.0
Bragg	0.50	VBC	170.47	6.59	110.3
D75 Ind	0.50	VBC	139.15	4.02	90.0
D75 Ind	1.00	VBC	117.30	4.85	75.9
Control	-	FAW	115.30	3.07	100.0
Bragg	0.50	FAW	152.15	4.02	132.0
D75 Ind	0.50	FAW	223.68	4.88	147.0
D75 Ind	1.00	FAW	184.91	13.12	82.7

¹ Fraction: Control=artificial diet; Bragg=benzene fraction of susceptible cultivar; D75 Ind=petroleum ether fraction of resistant mite-damaged D75-10169 cultivar.

² Concentration=percent dry weight of diet.

³ Species: VBC=velvetbean caterpillar; FAW=fall armyworm.

APPENDIX B

Results of mixed function oxidase detoxication activities of the larvae of two noctuid species fed artificial diets containing soybean foliar extract fractions and commercial coumestrol (Eastman Kodak Co.). Cytochrome P-450 oxidase activity (pm/min/mg protein) was measured for the 10 crude midgut homogenates (Yu 1982). Each mean represents the average of two subsamples.

Frac ¹	Conc ²	Spp ³	Mean Activity	se	% of Control
Control	-	VBC	257.31	20.89	100.0
Coumestrol	0.250	VBC	299.10	10.92	116.2
Coumestrol	0.500	VBC	263.59	7.83	102.4
Coumestrol	1.000	VBC	305.93	27.28	118.9
Benzene	0.125	VBC	251.88	13.52	97.9
Benzene	0.250	VBC	226.90	7.20	88.2
Benzene	0.500	VBC	335.36	6.58	130.3
Control	-	FAW	229.92	2.18	100.0
Coumestrol	0.250	FAW	172.71	2.05	75.1
Coumestrol	0.500	FAW	216.01	6.11	94.0
Coumestrol	1.000	FAW	228.46	6.24	99.4
Benzene	0.125	FAW	207.82	9.70	90.4
Benzene	0.250	FAW	171.82	12.89	74.7
Benzene	0.500	FAW	228.96	3.60	99.6

¹ Fraction: Control=artificial diet; Coumestrol (Eastman Kodak Co.; Benzene=susceptible (Bragg) fraction.

² Concentration=percent dry weight of diet.

³ Species: VBC=velvetbean caterpillar; FAW=fall armyworm.

APPENDIX C

Results of mixed function oxidase detoxication activities of the larvae of three noctuid species fed artificial diets containing soybean foliar extract fractions. Cytochrome P-450 oxidase activity (pm/min/mg protein) was measured for the 10 crude midgut homogenates (Yu 1982). Each mean for the FAW and CL species represents the average of two subsamples, whereas the VBC means represent 2 replicates, with two subsamples each.

Frac ¹	Conc ²	Spp ³	Mean Activity	se	% of Control
Control	-	VBC	111.22	1.79	100.0
Ace 1-3	0.5	VBC	40.86	3.31	36.7
Ace 1-3	1.0	VBC	55.07	4.23	49.5
Ace 21-23	0.5	VBC	110.07	3.74	99.0
Control	-	FAW	78.14	2.54	100.0
Ace 1-3	0.5	FAW	109.67	0.83	140.4
Ace 1-3	1.0	FAW	209.43	7.95	268.0
Ace 21-23	0.5	FAW	116.50	2.70	149.1
Control	-	CL	156.45	27.66	100.0
Ace 1-3	0.5	CL	397.92	11.18	254.3
Ace 1-3	1.0	CL	814.98	13.12	520.9
Ace 21-23	0.5	CL	556.31	10.44	355.6

¹ Fraction: Acetone fractions from Bragg benzene fraction.

² Concentration=percent dry weight of diet.

³ Species: VBC=velvetbean caterpillar; FAW=fall armyworm; CL=cabbage looper.

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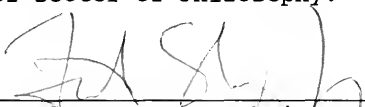
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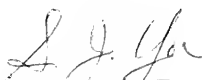
BIOGRAPHICAL SKETCH

Gregory S. Wheeler was born August 25, 1955 in Seattle, Washington to Mr. and Mrs. Kenneth E. Wheeler. He was raised, until the age of 14, in the neighboring suburbs where, among the Douglas Firs and the seashore, he developed a respect for natural history. Following high school graduation at East Valley High in Yakima, Washington, he graduated from Central Washington University with a B.A. degree in biology. Directly following graduation he entered the Peace Corps where he worked for 3 years developing integrated pest management programs in field beans and corn in Nicaragua and Honduras, Central America. Upon returning to the United States he worked for a year and a half at Colorado State University on various pest management programs. However, the desire to pursue advanced degrees was too strong and in the long winter of 1981 he began what was to be a 2 year M.S. program in entomology on the biocontrol of rangeland weeds at the University of Idaho. Following a year as a research supervisor in Honduras at the Pan American Agricultural School, he entered a Ph.D. program in the Spring of 1985, and will continue working, following graduation, as a postdoctoral fellow on the chemical/nutritional ecology of herbivores.

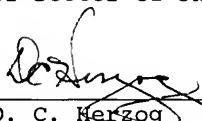
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F. Slansky, Jr., Chair
Associate Professor of
Entomology and Nematology

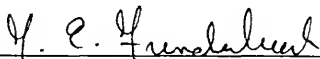
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S. J. Yu
Professor of Entomology and
Nematology

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.


D. C. Herzog
Professor of Entomology and
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I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.


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